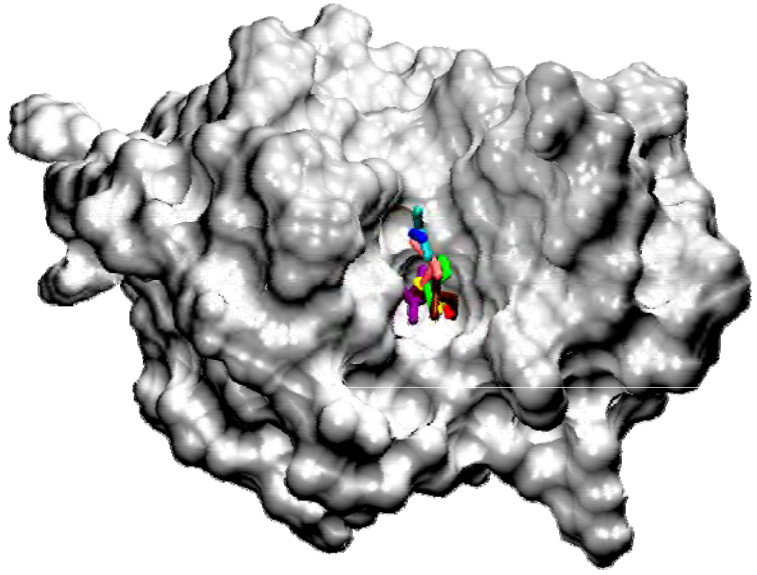
Mapping of proteins for the binding of functional groups from xenobiotics

Sandor Vajda
Department of Biomedical Engineering, Boston University



Application of drug design methods to the analysis of protein-xenobiotics interactions

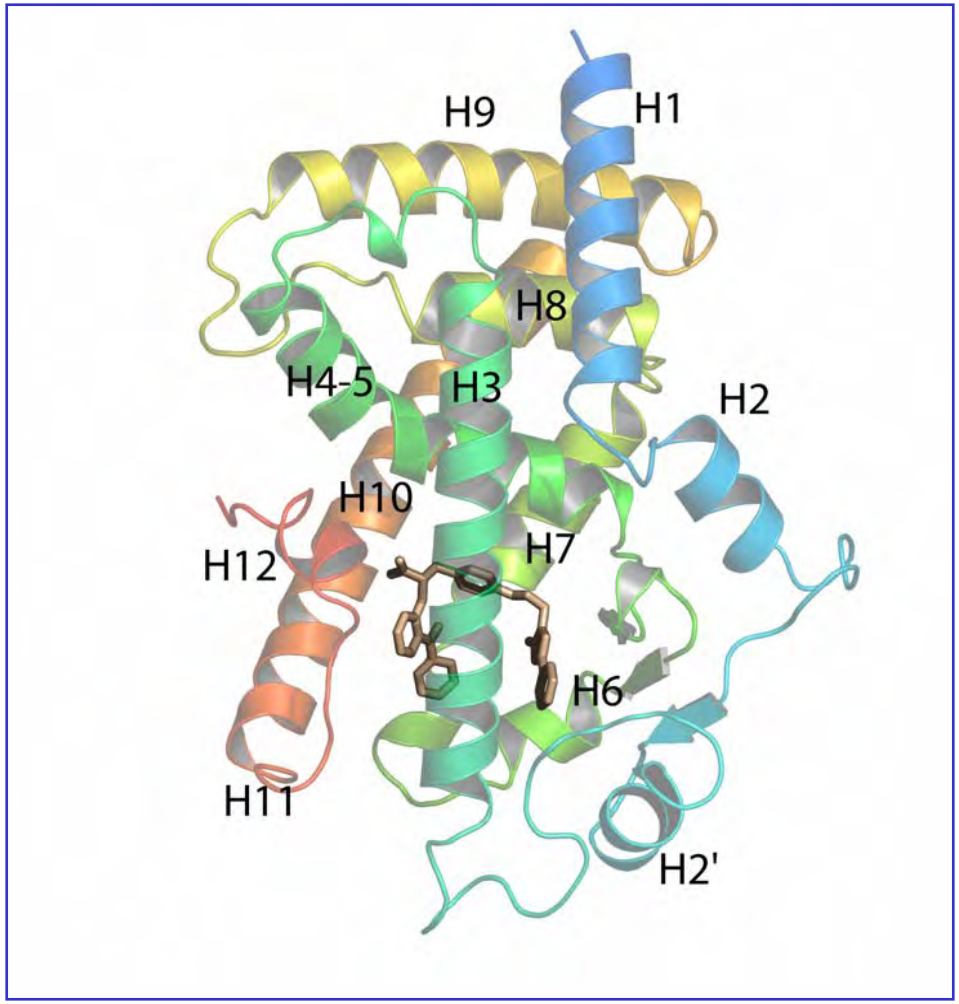
1. Traditional approach:
   Docking and virtual screening
   Example: Binding of phthalate monoesters to PPARγ

2. New approach "borrowed" from fragment-based drug design:
   Protein mapping
   Example: Mapping of PPARγ

3. Ideas and dreams with potential application to toxicology

Peroxisome Proliferator Activated Receptor (PPAR)

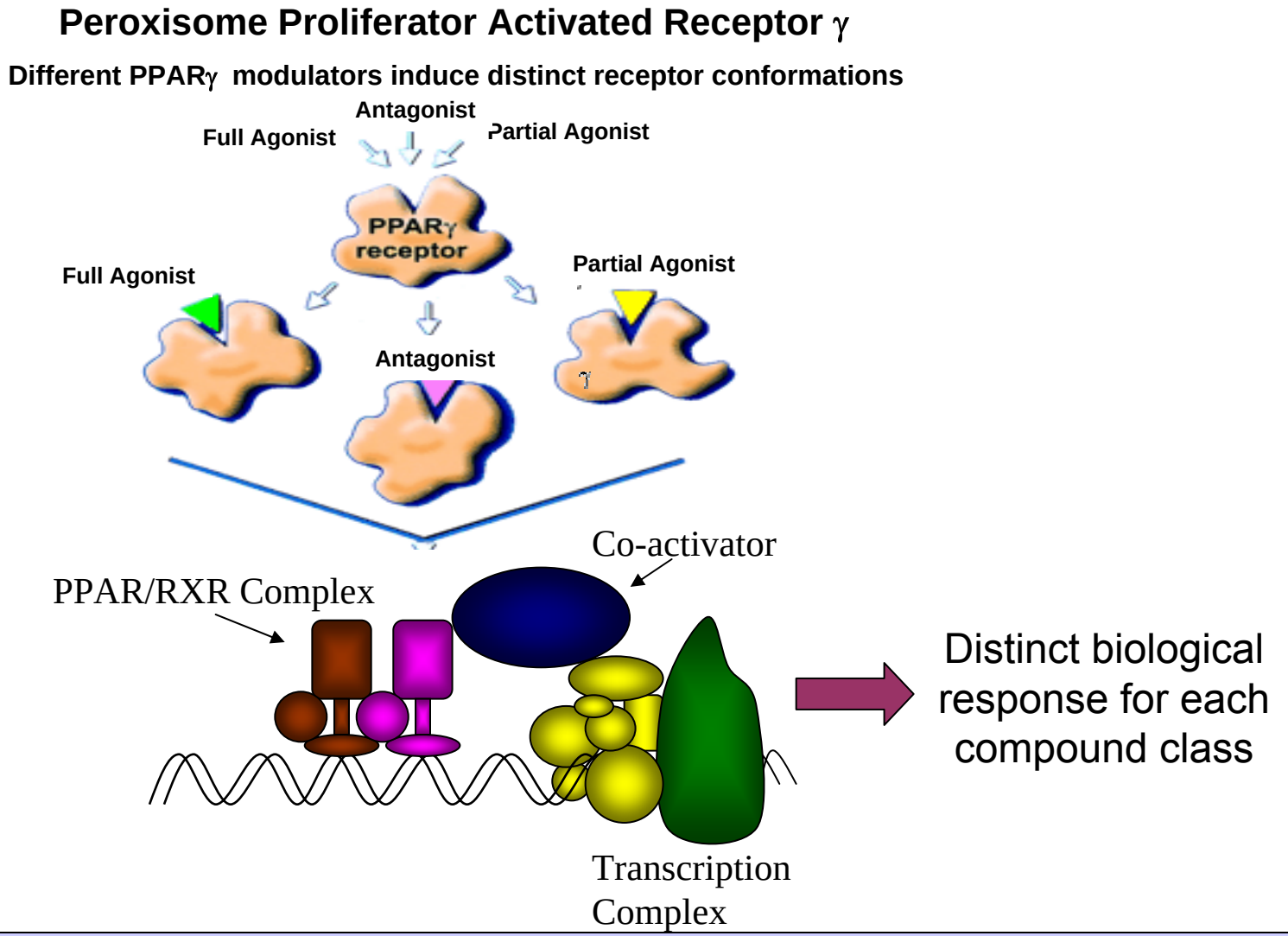
PPAR isotypes: α, δ, γ



- Ligand-activated transcription factor
- Member of the nuclear receptor superfamily
- Plays an important role in:
  - adipogenesis and
  - glucose homeostasis.

S. Vajda, 2007

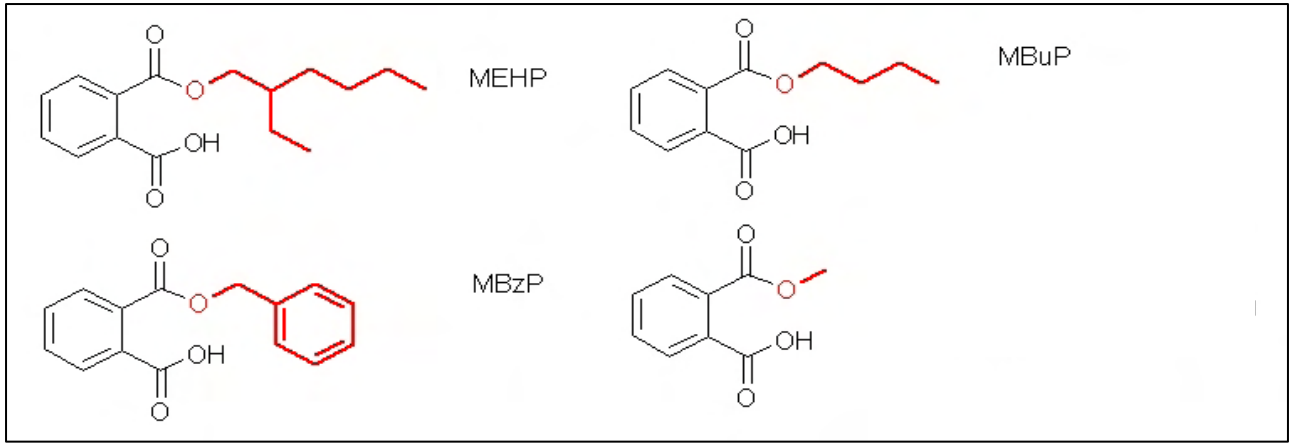
Applications to the PPARγ ligand-binding domain.



Adapted from Olefsky JM & Saltiel AR, TEM (2000) 11, 362-367

S. Vajda, 2007

Some phthalate monoesters are (weak) PPARγ agonists



	Increased activity reported upon binding for the following;
MEHP	**α Mouse (Human), EC_{50}= 0.6 µM (3.2 µM)** **γ Mouse (Human), EC_{50}=10.1 µM (6.2 µM)**
MBzP	**α Mouse (Human), EC_{50}=21uM (30 µM)** **γ Mouse (Human), EC_{50}=75 – 100 µM**
MBuP	**α Mouse , EC_{50}= 63 µM**
Monomethyl	**No activity**

S. Vajda, 2007

1. Binding of phthalates to PPARγ

- 5 – 10 million tons of phthalate plasticizers annually.
- found in toys, water containers, and even plastic wrap
- virtually all medical devices made from PVC

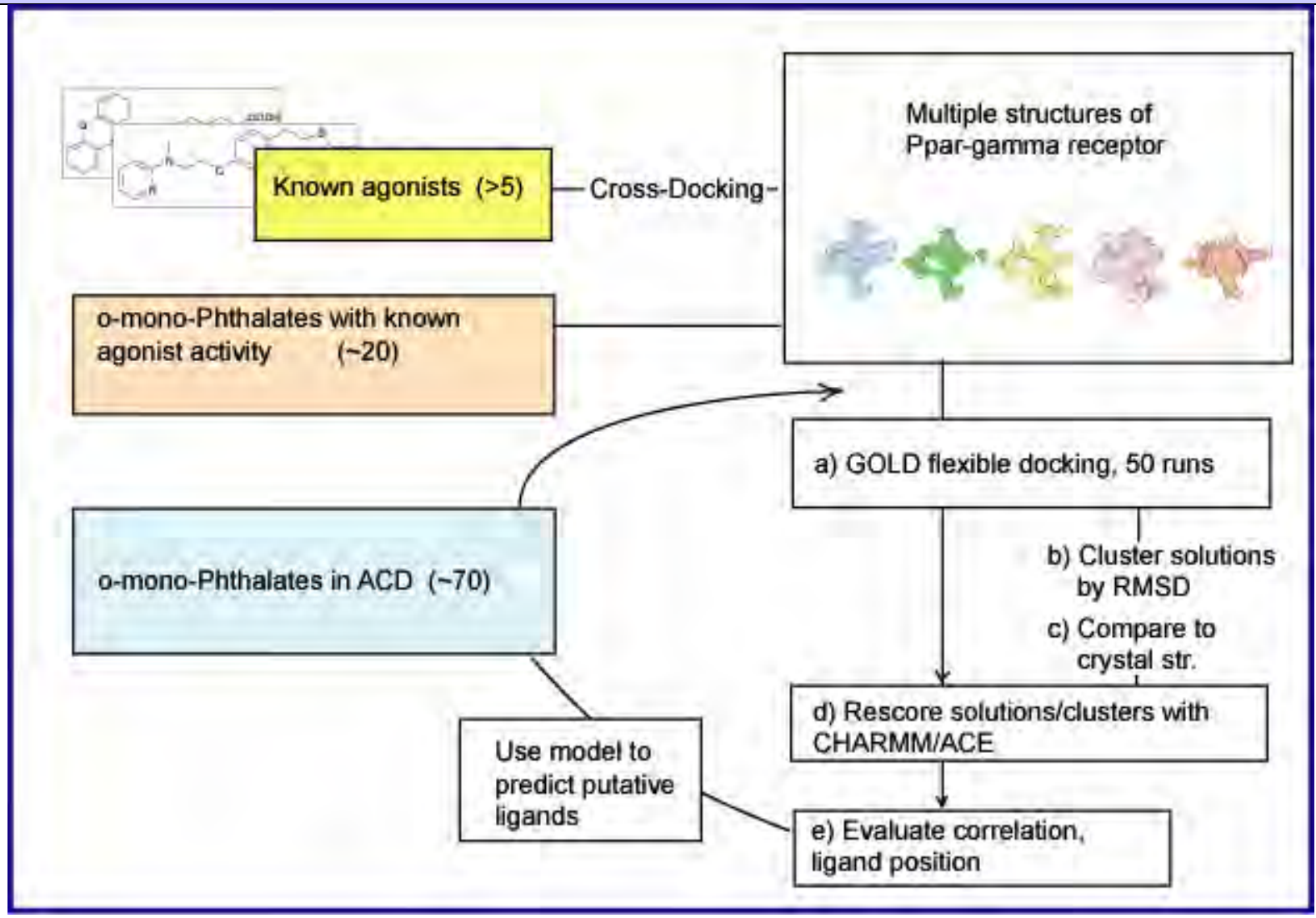
Effects include:

- reproductive toxic effects and endocrine disruption
- carcinogenicity
- acute toxicity (MEHP)

Goal: To identify all phthalate monoesters likely to affect PPARγ

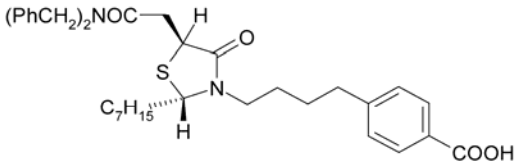
Kaya, T., Mohr, S. C., Waxman, D. J. & Vajda, S. (2006). Computational screening of phthalate monoesters for binding to PPARγ. *Chem Res Toxicol* **19**, 999-1009.

Method validation, docking, and screening

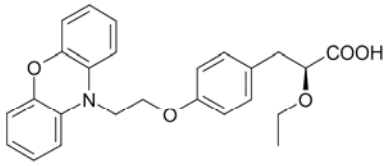


S. Vajda, 2007

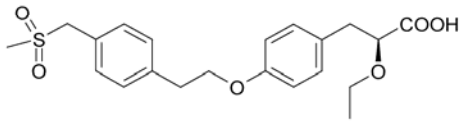
Validation step 1: Docking of known PPARγ agonists



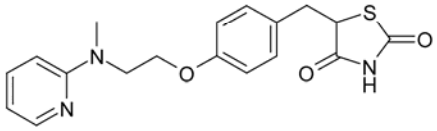
GW0072 (Partial agonist), PDB code 4prg



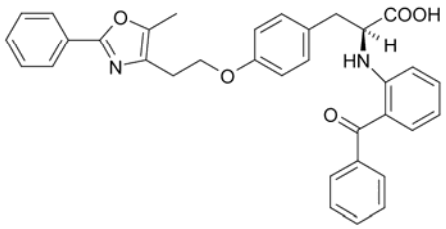
Ragaglitazar, PDB code 1nyx



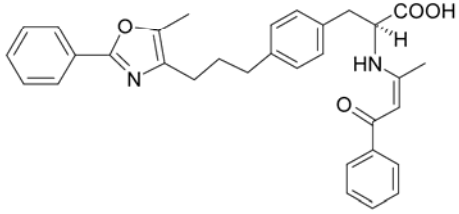
AZ242 (Tesaglitazar), PDB code 1i71



Rosiglitazone, PDB code 2prg and 1fm6



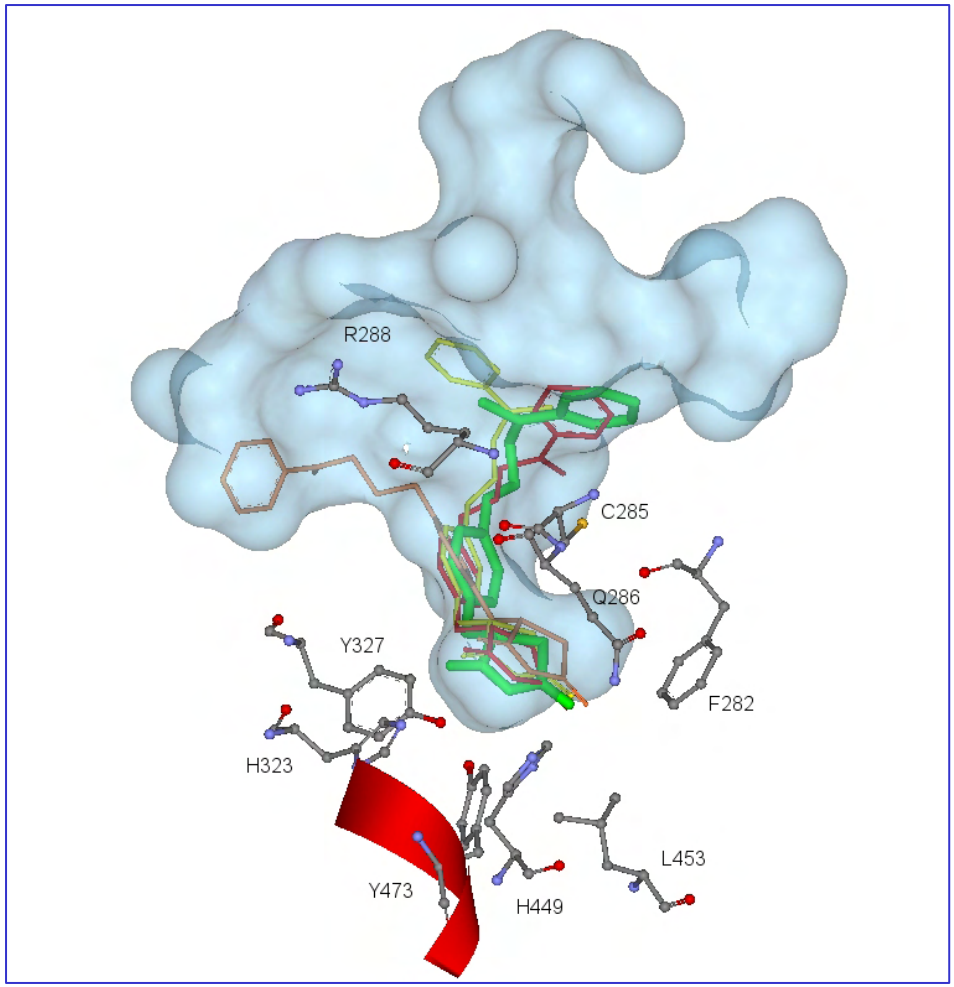
GI262570 (Farglitazar), PDB code 1fm9



GW409544, PDB code 1k74

S. Vajda, 2007

Validation Step 1: Docking of known PPARγ agonists

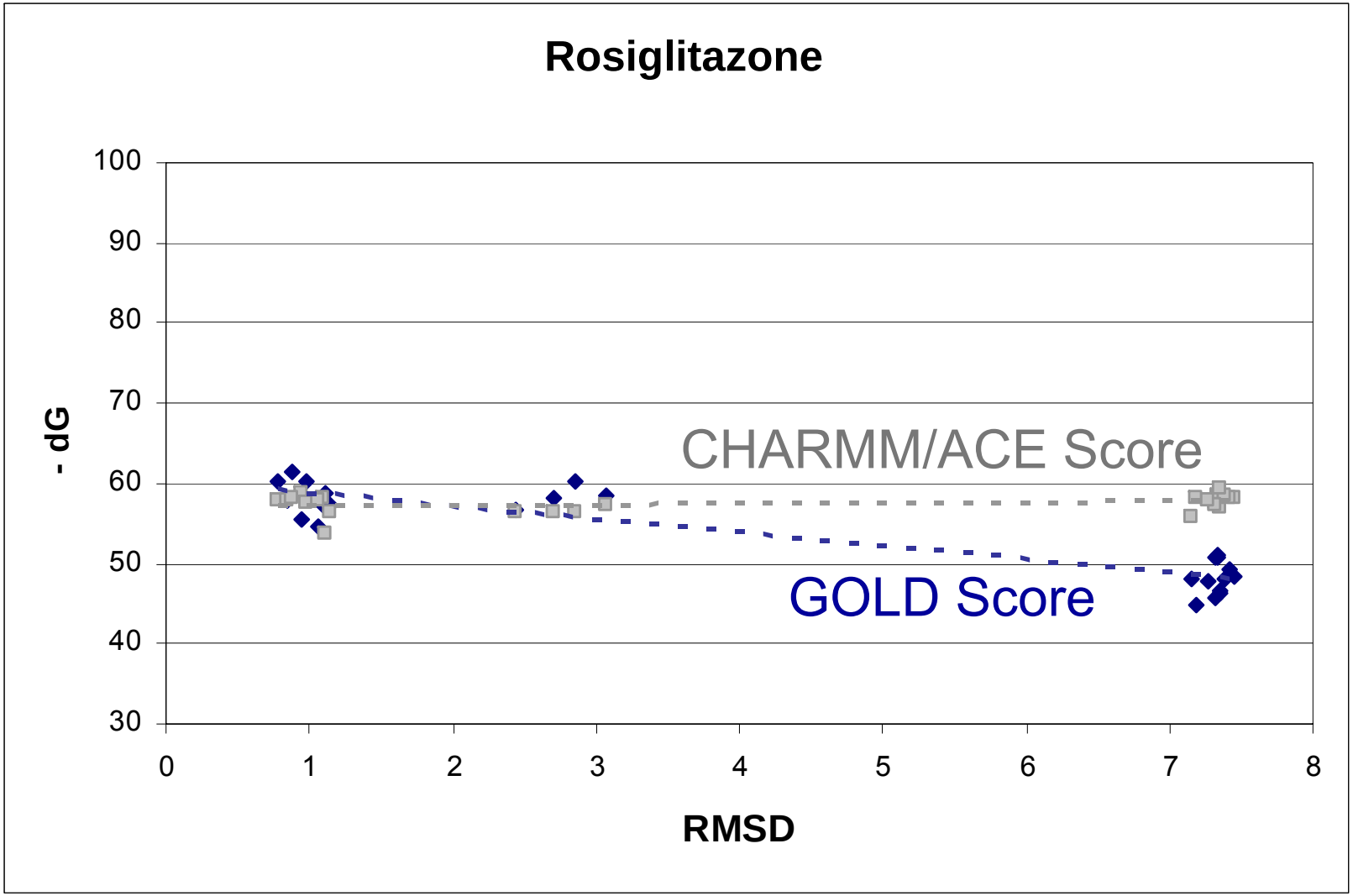


Crystallographic studies identify several residues as part of the H bonding pattern present in "AF-2" region, which accounts for the majority of the polar interactions between agonist ligands polar head groups (e.g. TZD, or carboxylic acid group) and the pocket (His323, Y473, H449).

Crystal structure orientation of Rosiglitazone, representative orientations from docked solutions, Cluster1, Cluster2, Cluster3

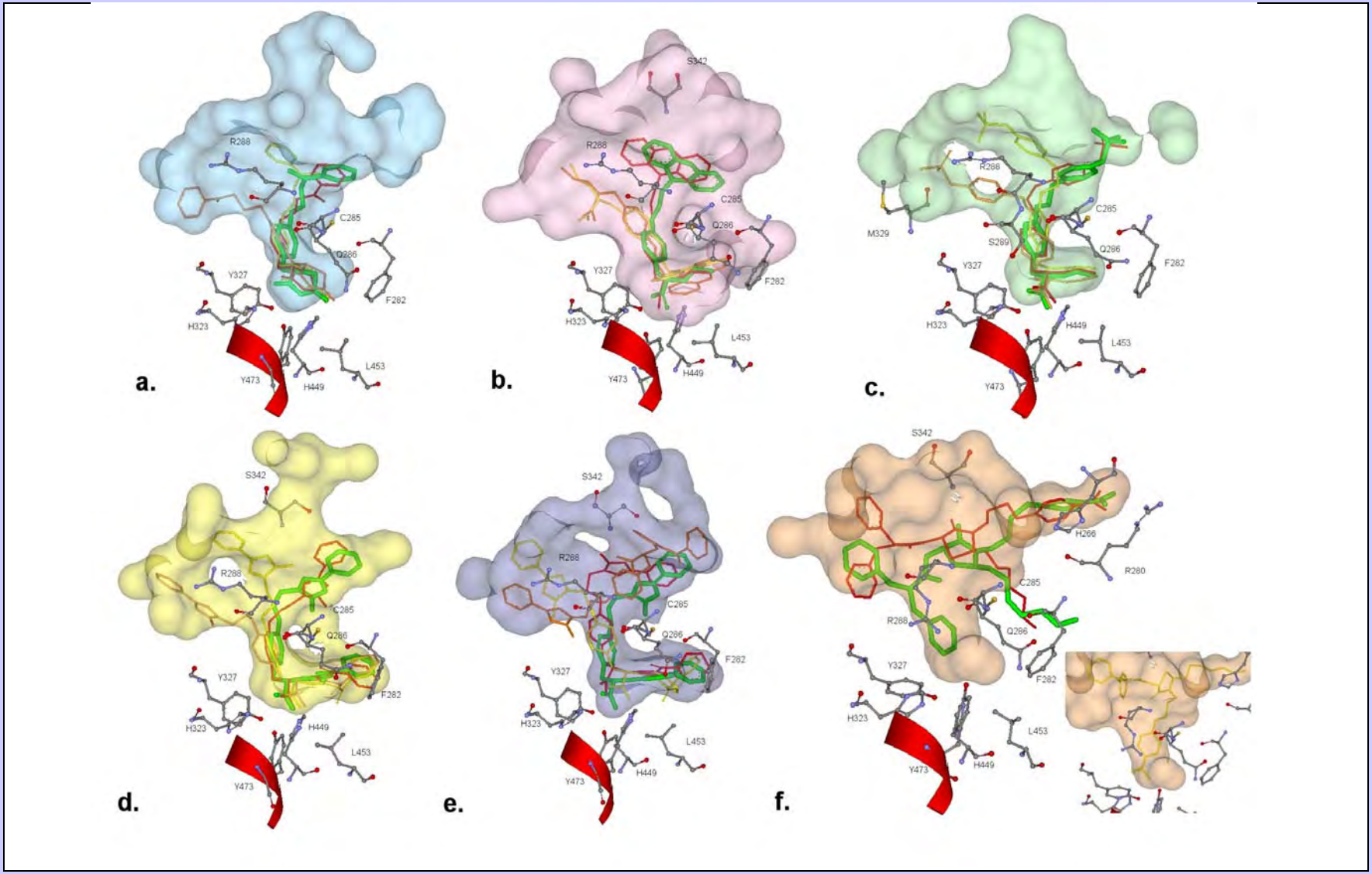
S. Vajda, 2007

Discrimination of docked orientations using GOLD *vs.* CHARMM/ACE



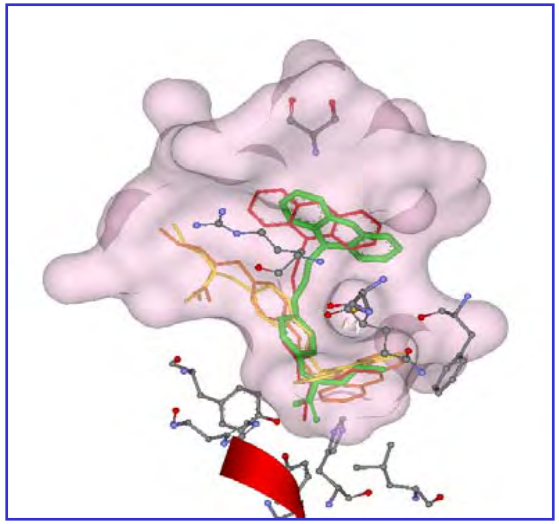
S. Vajda, 2007

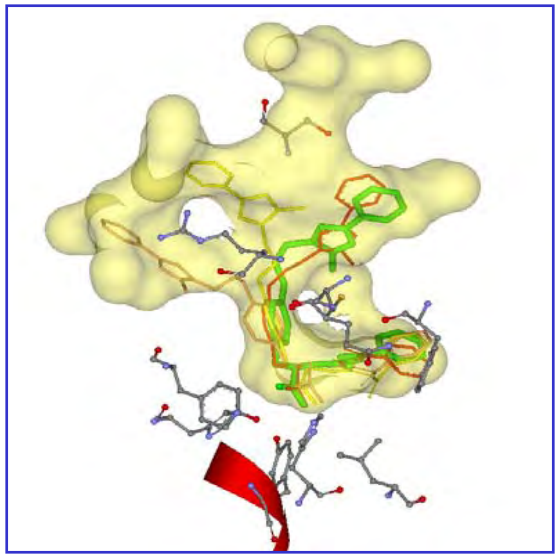
Docked orientations of agonists, crystal structure is shown in green

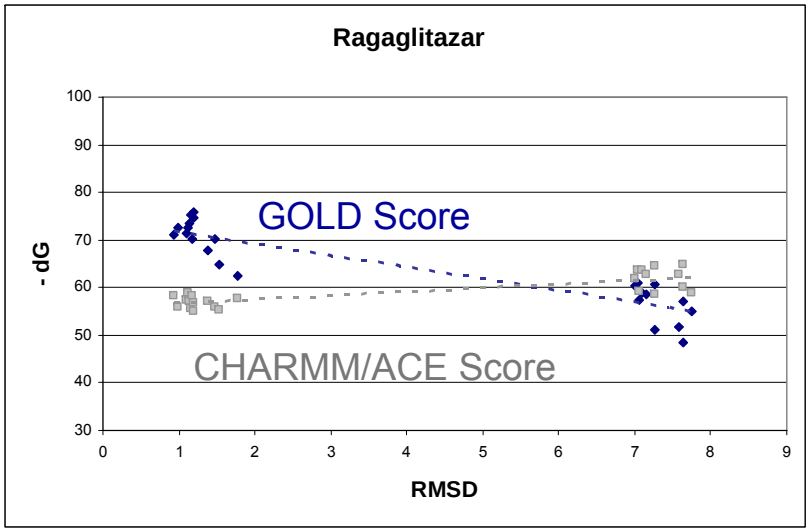


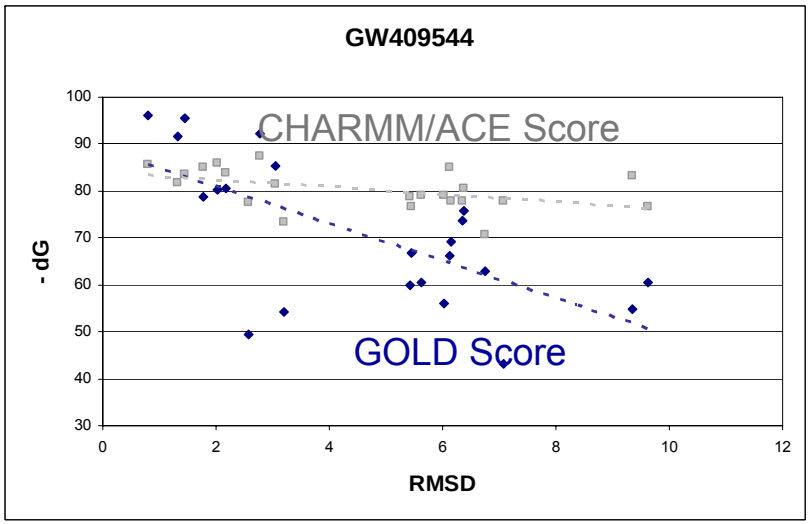
S. Vajda, 2007

Near-native poses are generated, but discrimination is difficult



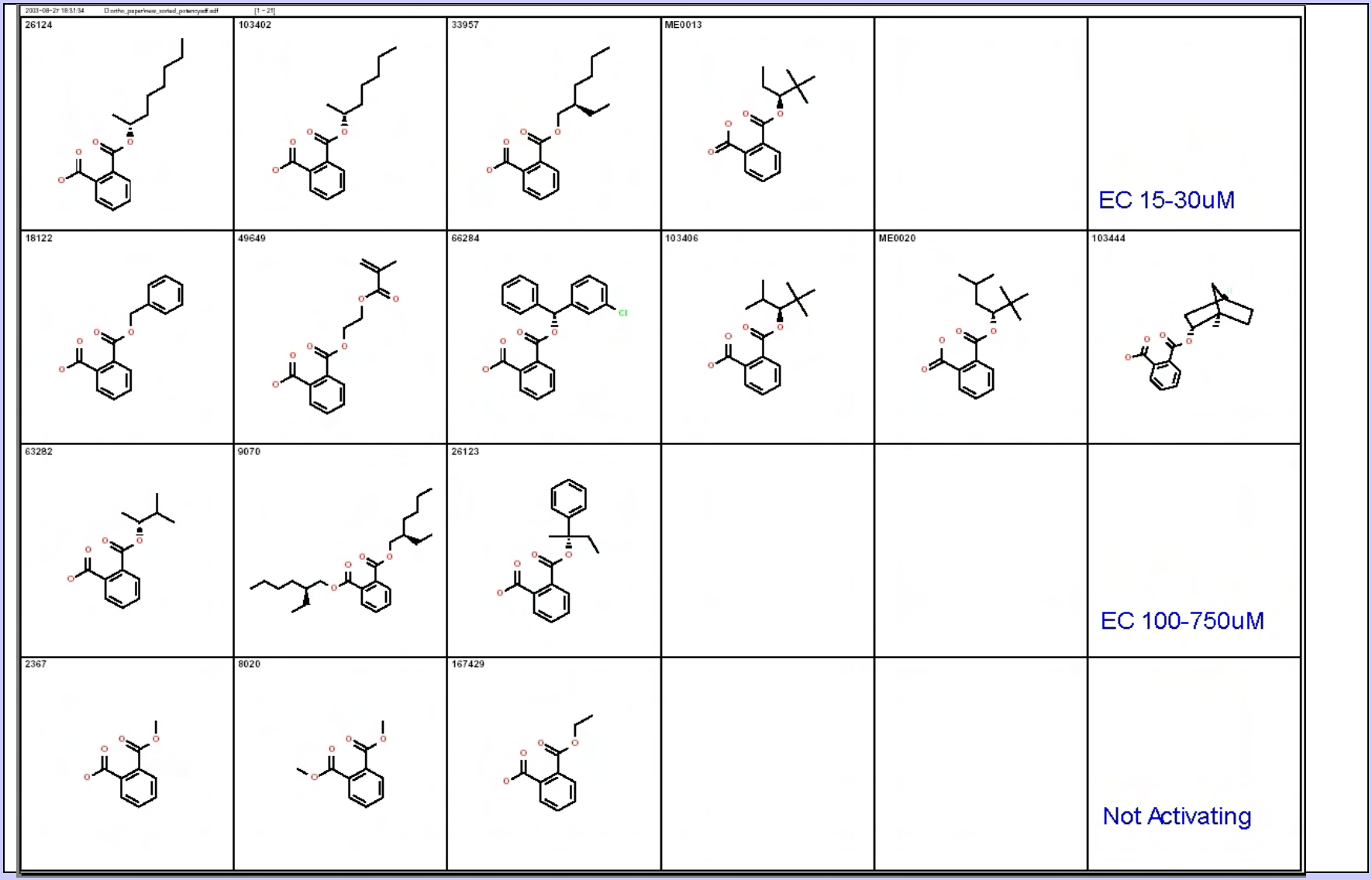






S. Vajda, 2007

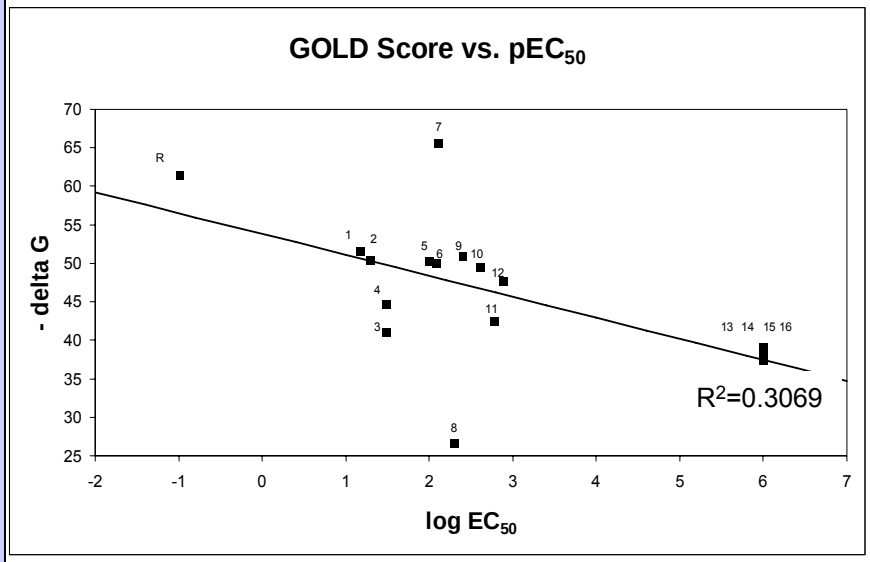
Validation Step 2: Prediction of phthalate binding to PPARγ

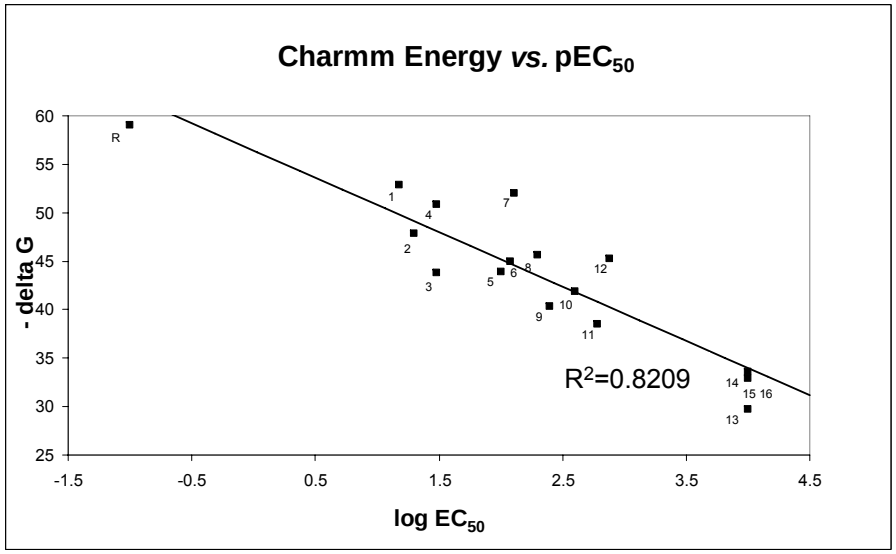


Lampen *et al.*, 2003, Tox.&Appl.Phar.

S. Vajda, 2007

Correlation between experimentally determined transactivation values (EC_{50}) for ortho-monophthalates and ΔG ,GOLD vs. CHARMM/ACE

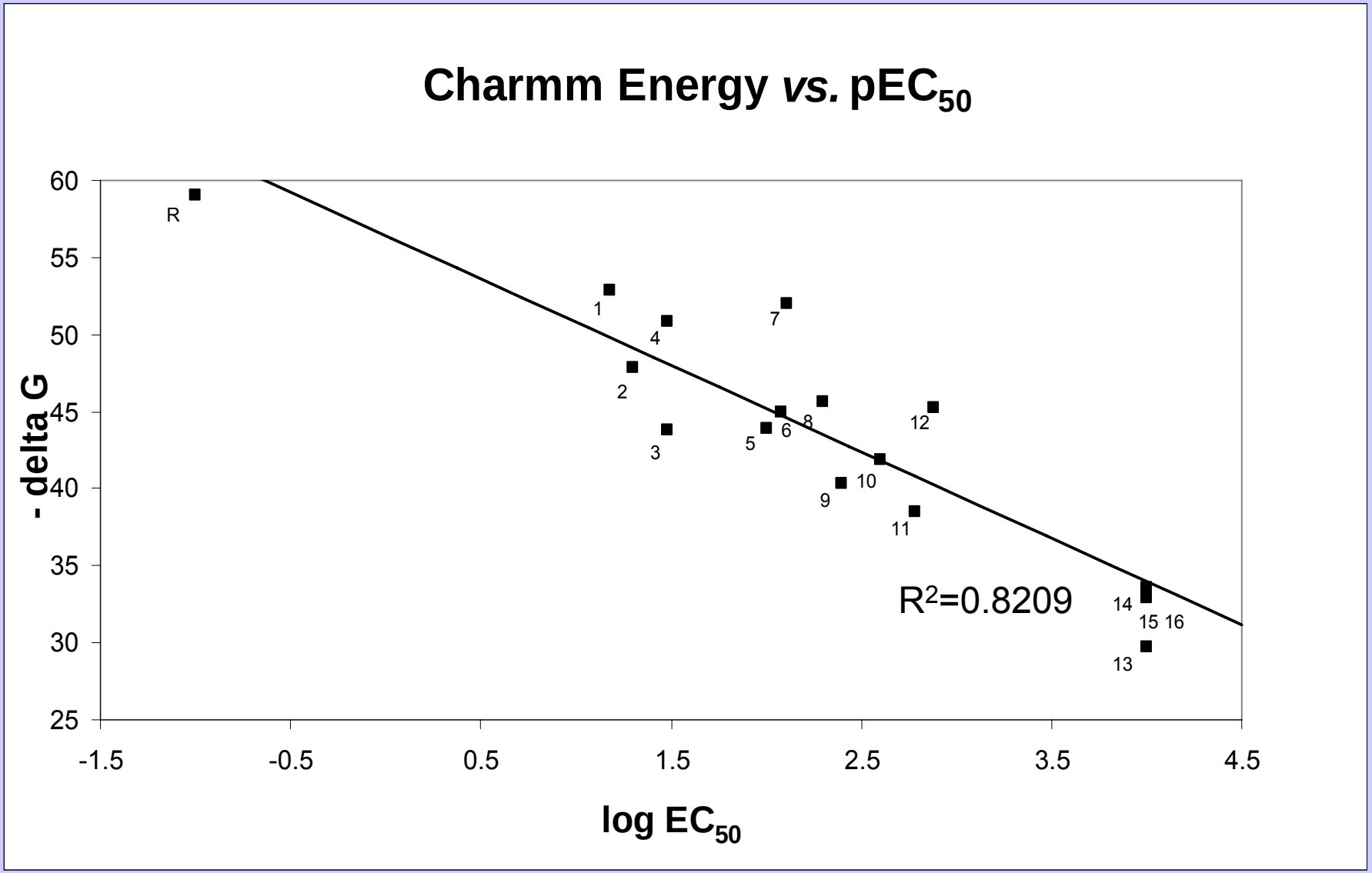




Correlation is improved using the binding free energy calculated with CHARMM/ACE force field, as opposed to using GOLD scoring function .

S. Vajda, 2007

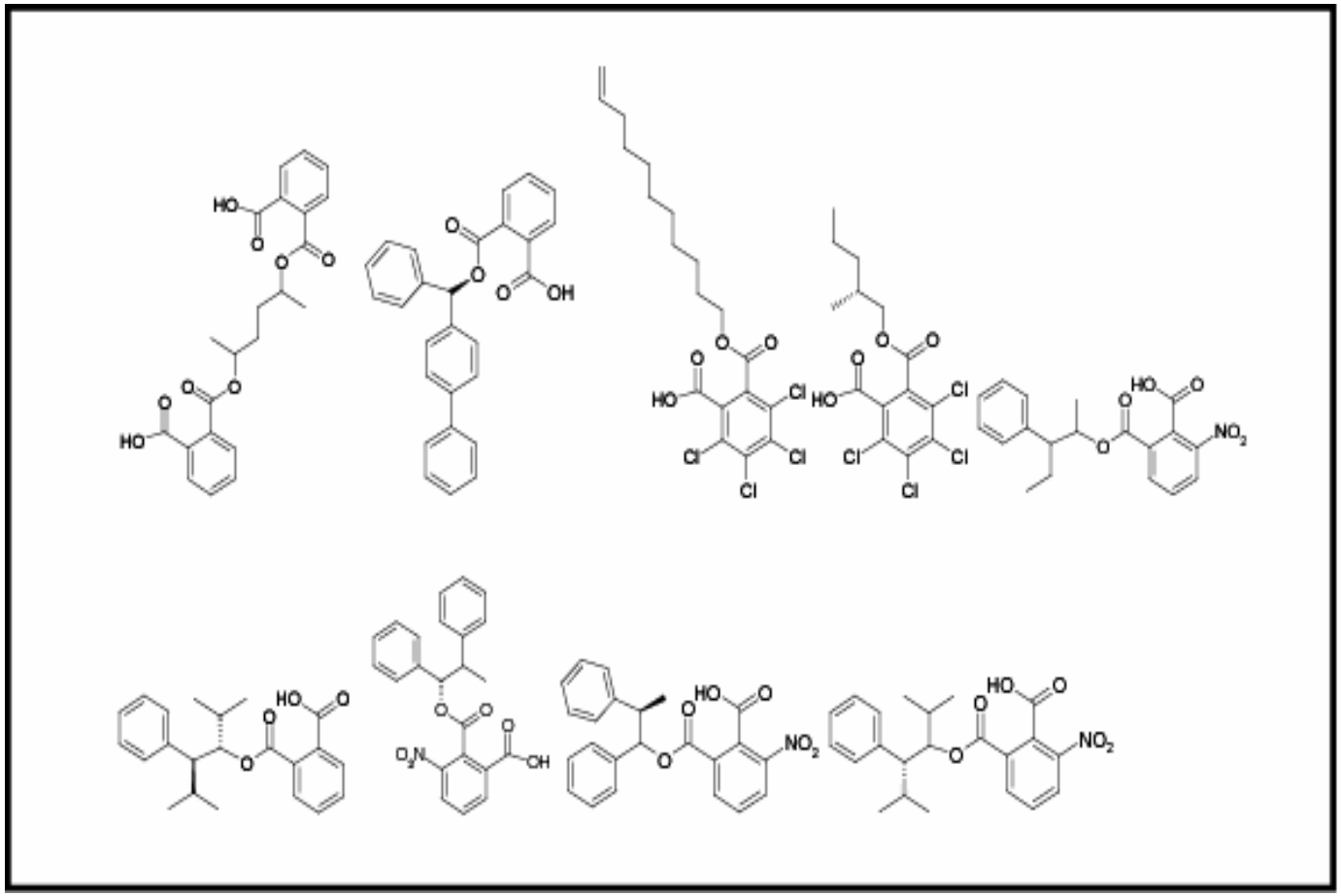
MM/GBSA (CHARMM/ACE) predicts transactivation



S. Vajda, 2007

Screening of 73 ortho-mono-phthalates in the ACD for PPARγ binding

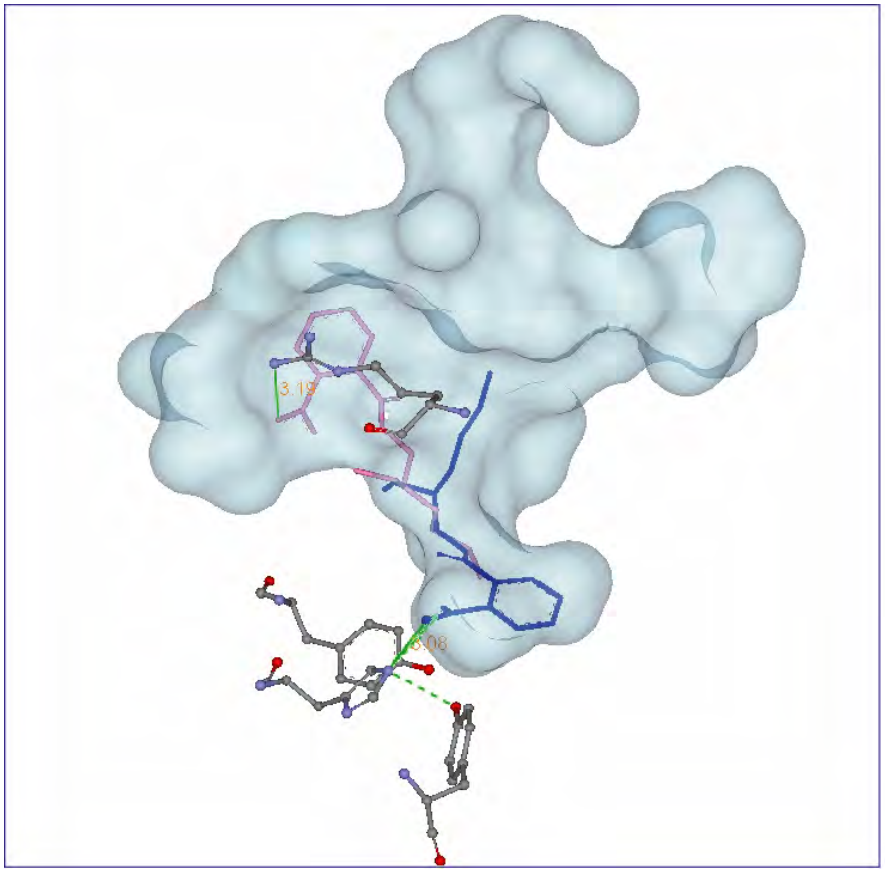
Using our method we predicted a number of other ortho-mono-phthalates besides already known trans-activating phthalates.



S. Vajda, 2007

Docked o-monophthalate orientations: no mechanistic insight

Two major docked orientation clusters were observed for activating ortho-monophthalates:



Cluster (a) Agonist-like binding: Exhibits interactions similar to known PPAR agonists, where H-bonding with AF-2 residues is visible. (absent for non-activating phthalates)

Cluster (b) Partial agonist like binding: Orientations are placed in the center of the pocket, where -COO^- is H-bonded to R288. Alkyl chain positions varied in docked orientations.

The binding free energies of various orientations for a given ortho-mono-phthalate remained similar, unless H bonding was absent.

S. Vajda, 2007

Conclusions from the docking and screening studies

- Negative: Near-native structures are generated, but are difficult to discriminate from non-native poses (The GOLD docking score is slightly better than the more rigorous CHARMM/ACE score based on a MM/GBSA model).

  Limited mechanistic insight!

- Positive: The MM/GBSA (CHARMM/ACE) scores correlate well with transactivation data (a somewhat unexpected result).

- Docking becomes increasingly difficult if:

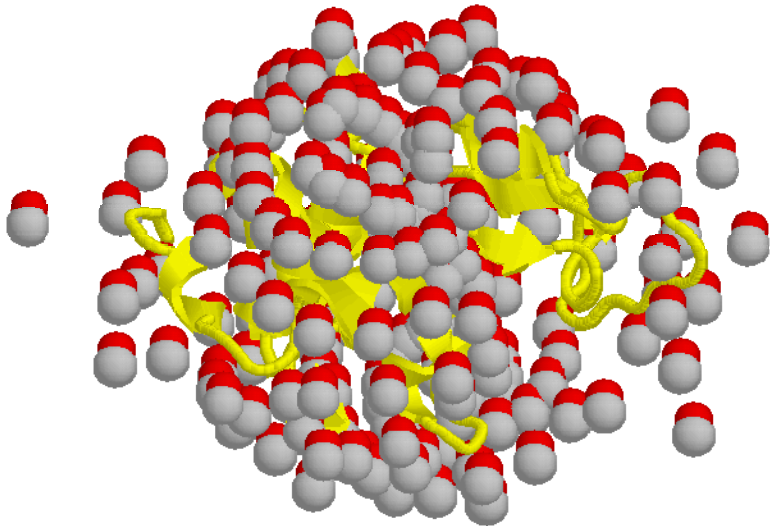
  (a) Degrees of rotational freedom increases

  (b) Substantial change in protein conformation occurs

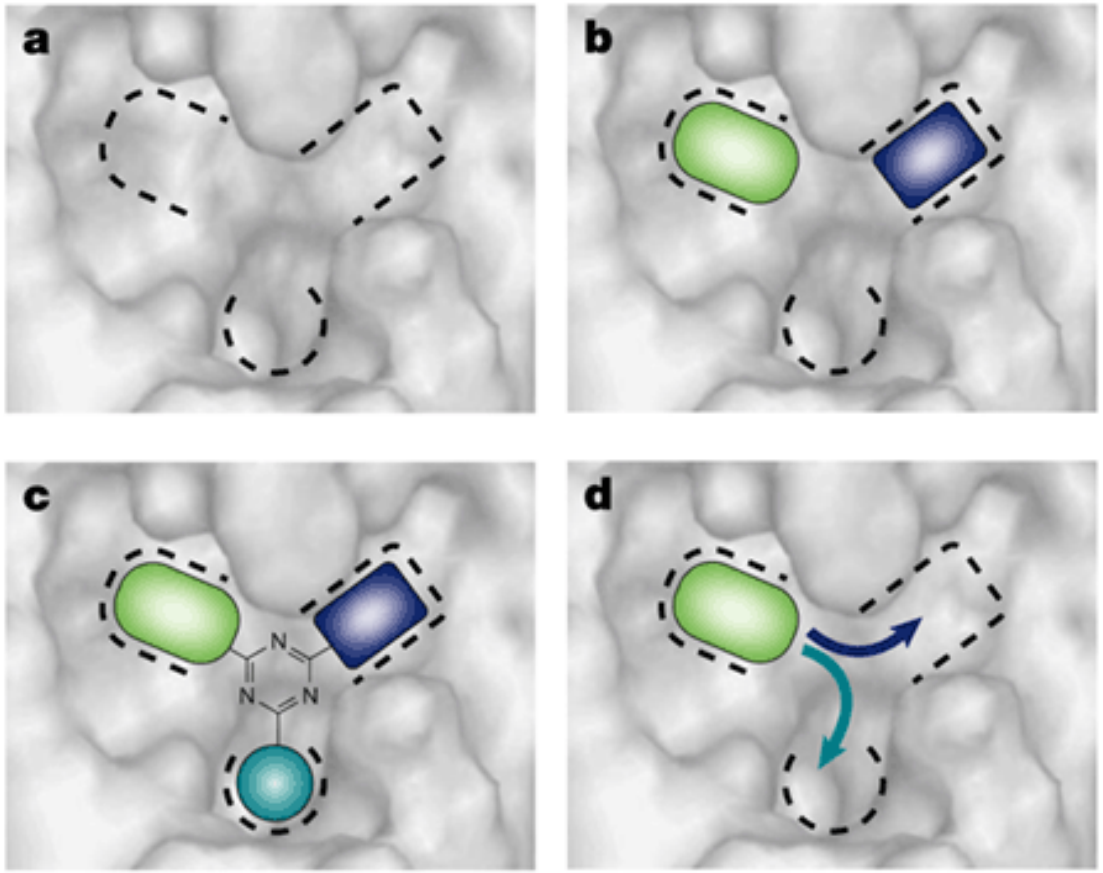
S. Vajda, 2007

2. Protein mapping

Mapping of a protein means surrounding it by molecular probes – small molecules or functional groups –in order to determine the most favorable binding positions.



Protein mapping in fragment-based drug design



a. A binding site comprising three pockets

b. Mapping of the binding site to find molecular fragments that bind in particular pockets

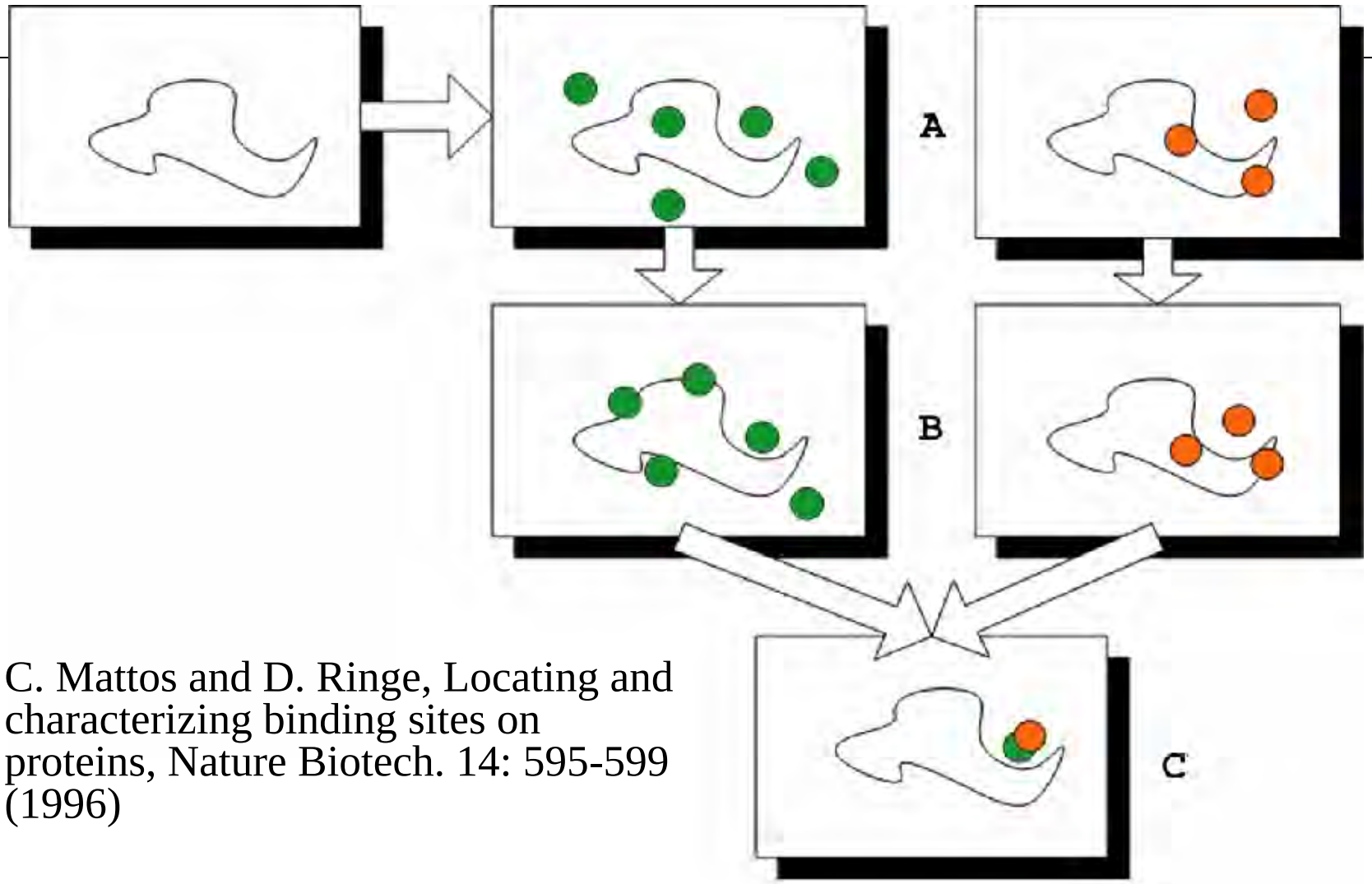
c. Concatenation of molecular fragments into a possible lead using a core template. A number of good binding fragments can be used at each position resulting in a combinatorial library

d. Building lead compounds by extending the best binding molecular fragment.

Nature Reviews Drug Discovery **1**; 45-54 (2002)

S. Vajda, 2007

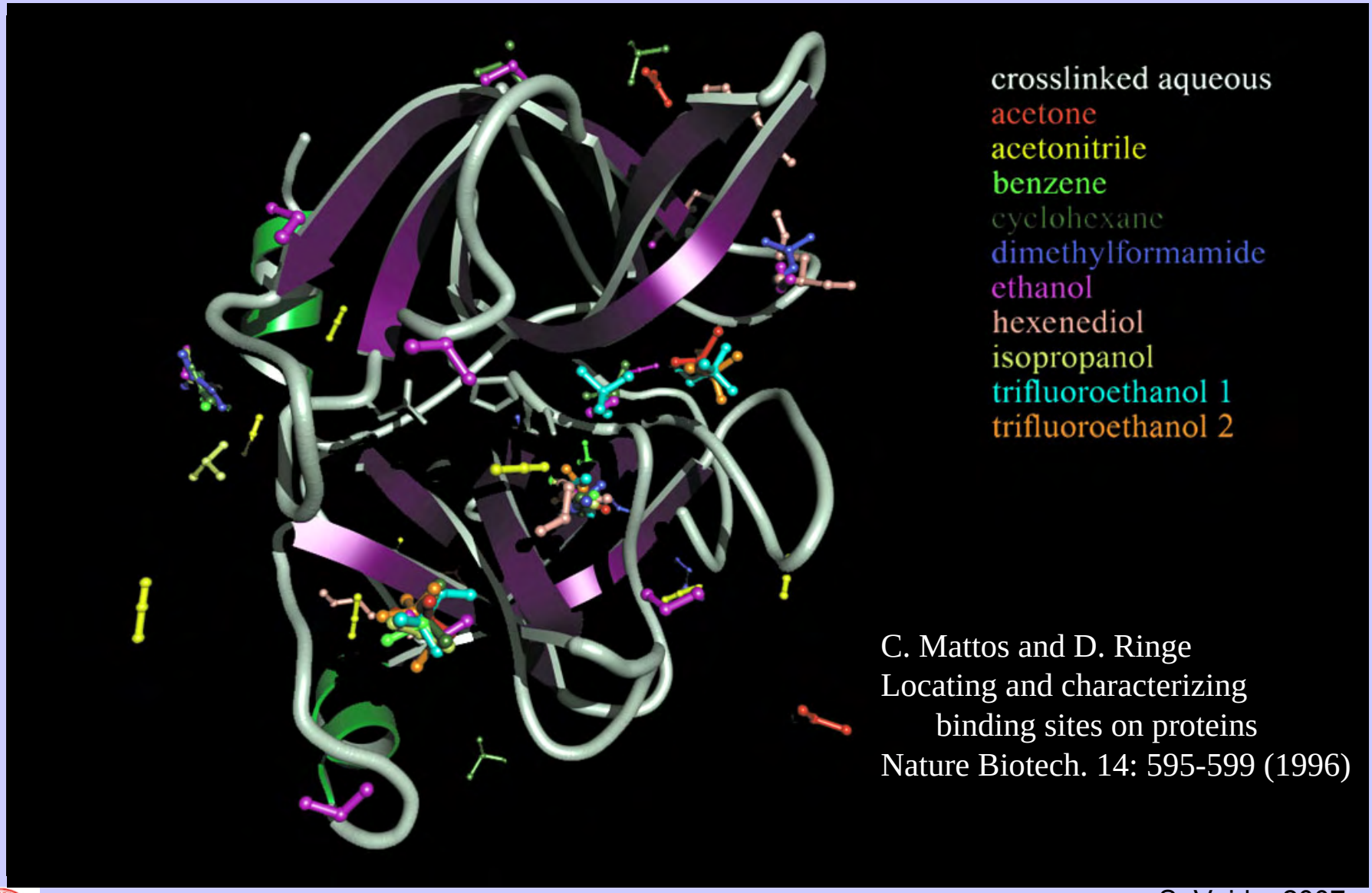
Mapping by Multiple Solvent Crystal Structures



C. Mattos and D. Ringe, Locating and characterizing binding sites on proteins, Nature Biotech. 14: 595-599 (1996)

S. Vajda, 2007

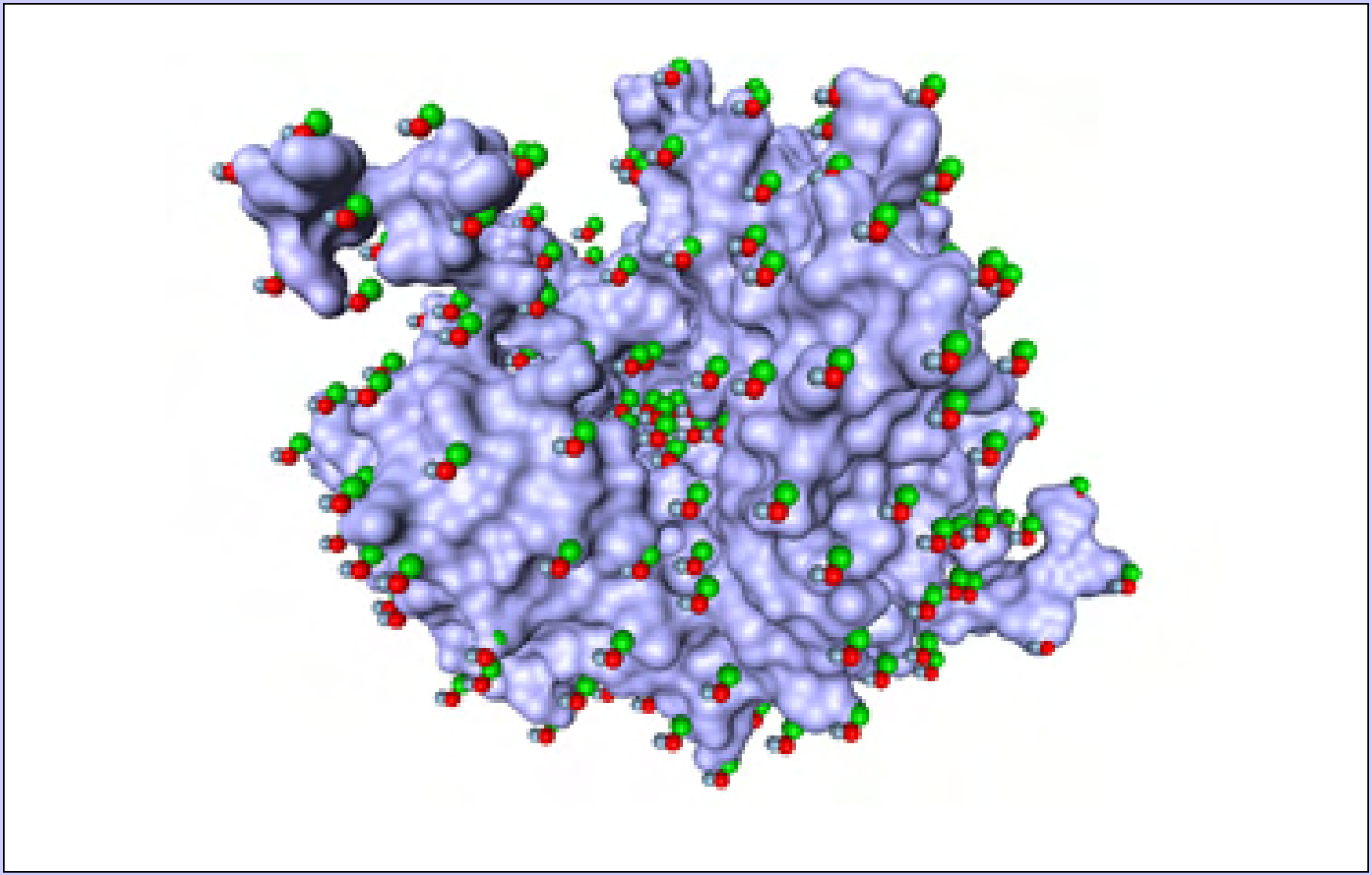
Multiple solvent crystal structures for elastase



C. Mattos and D. Ringe
Locating and characterizing binding sites on proteins
Nature Biotech. 14: 595-599 (1996)

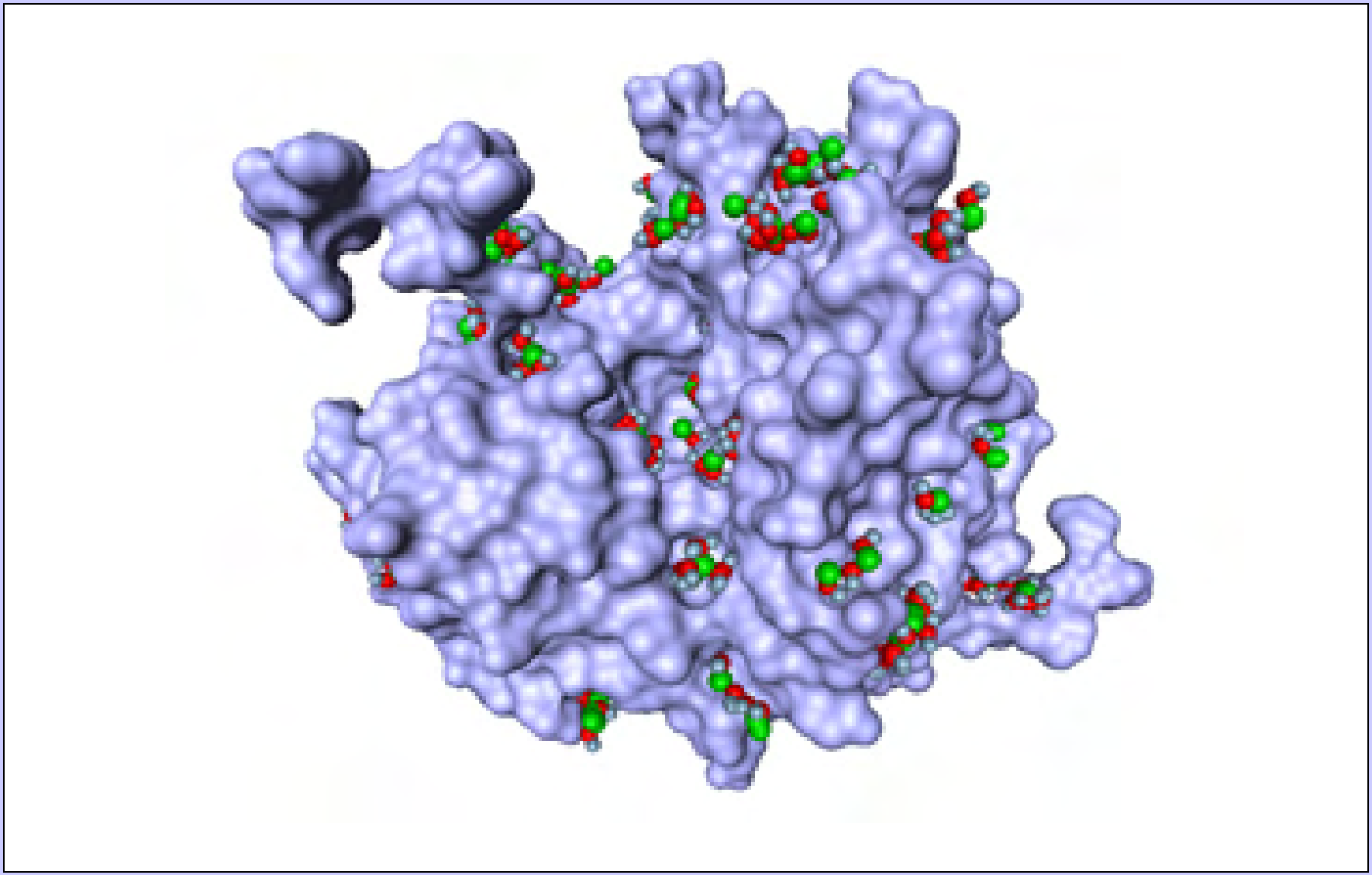
S. Vajda, 2007

Computational mapping - CS-Map Step 1: Placing the probes



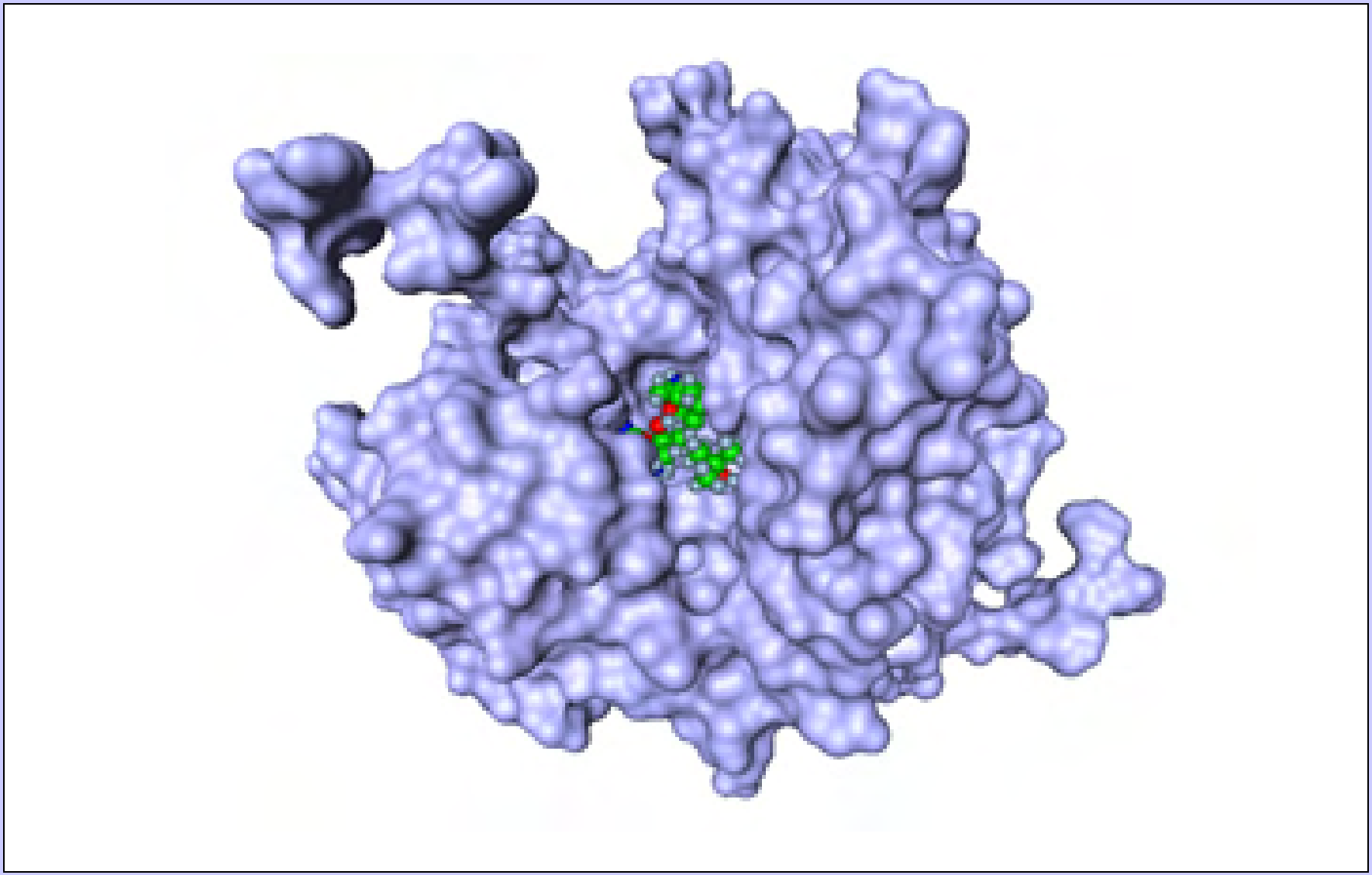
S. Vajda, 2007

CS-Map Step 2: Move the probes around to find binding positions



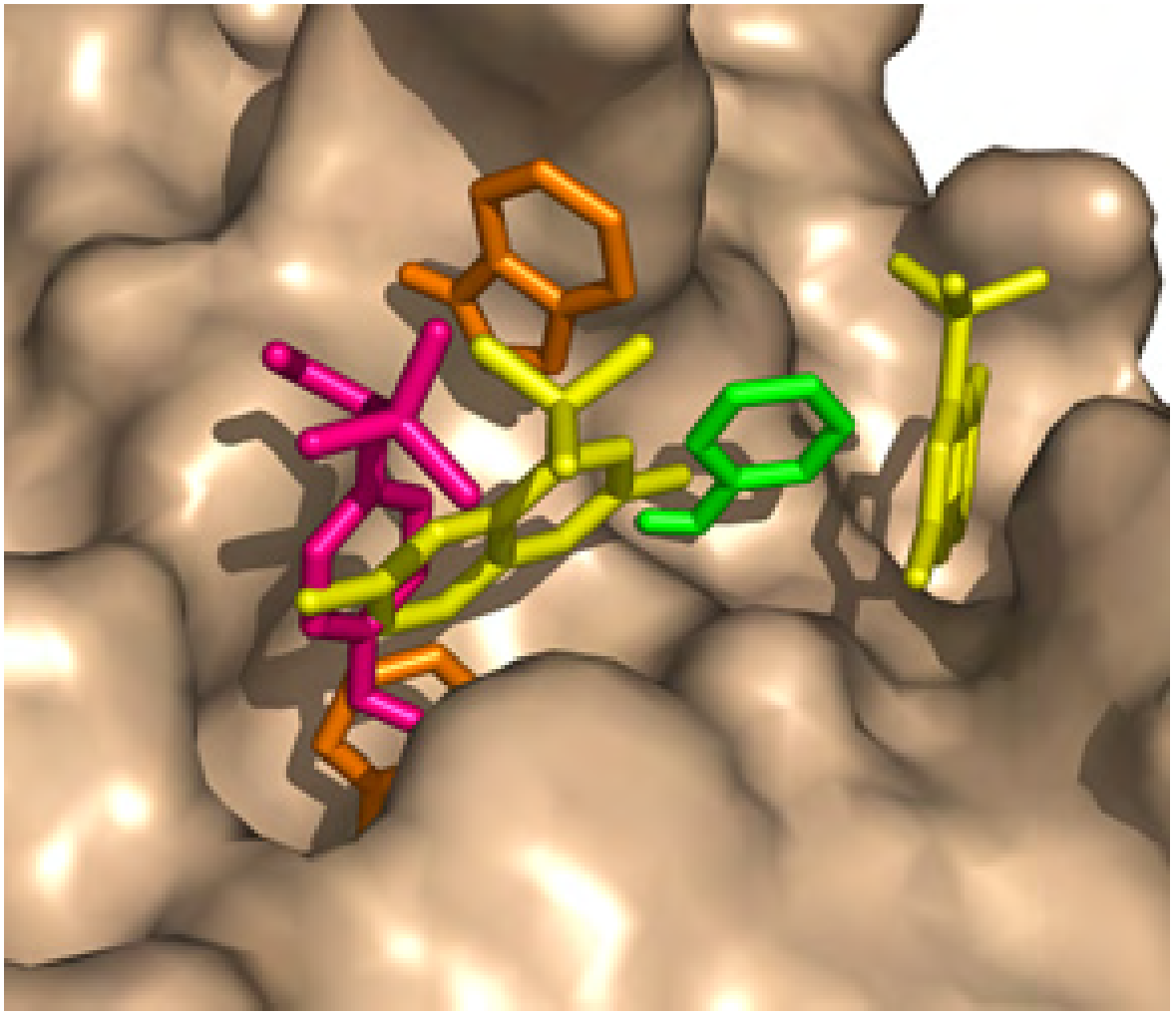
S. Vajda, 2007

CS-Map Step 3: Find low energy clusters of bound ligands



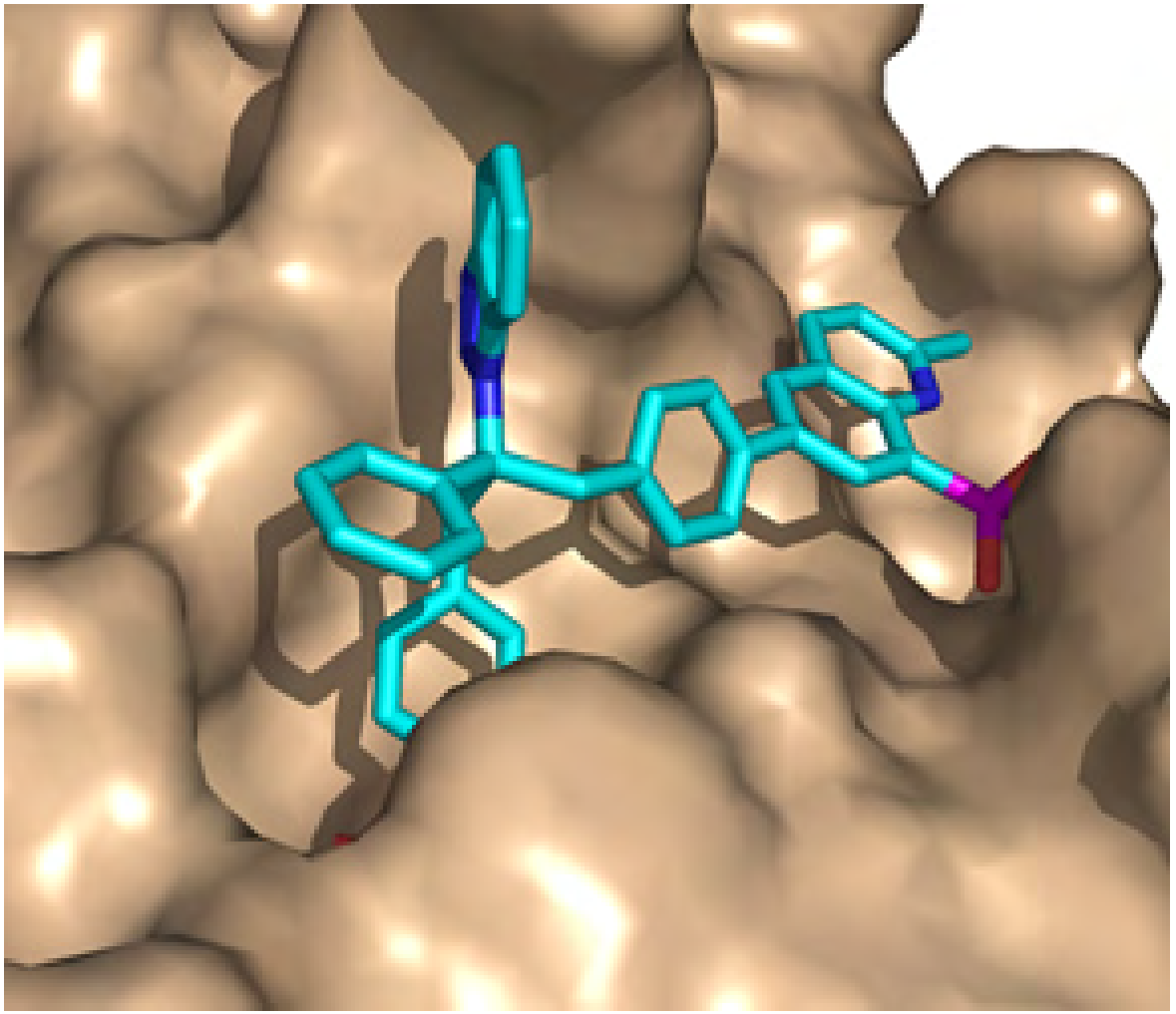
S. Vajda, 2007

CS-Map Step 4: Repeat mapping with a number of fragments



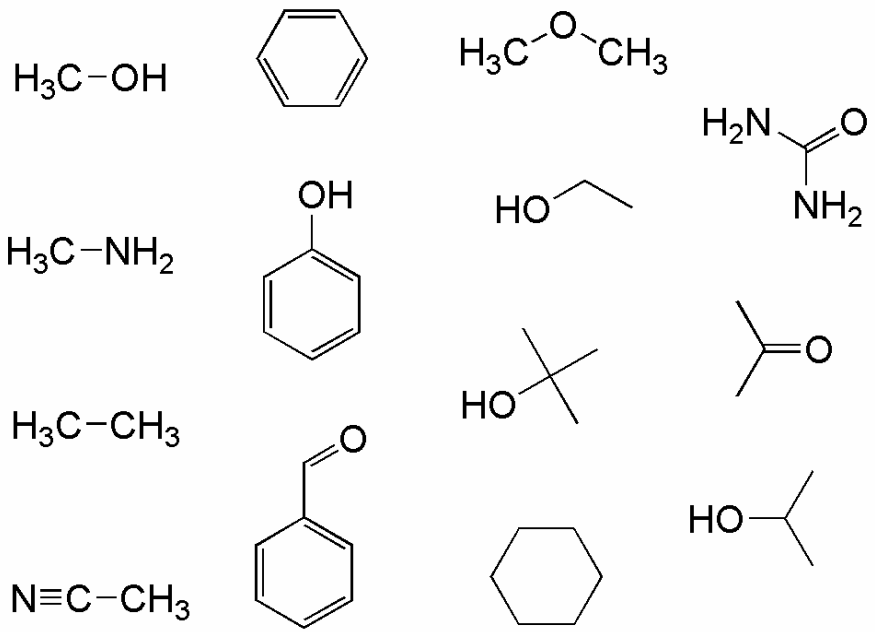
S. Vajda, 2007

CS-Map Step 5: (Combine fragment into ligand molecules)



S. Vajda, 2007

Initial probe set for mapping



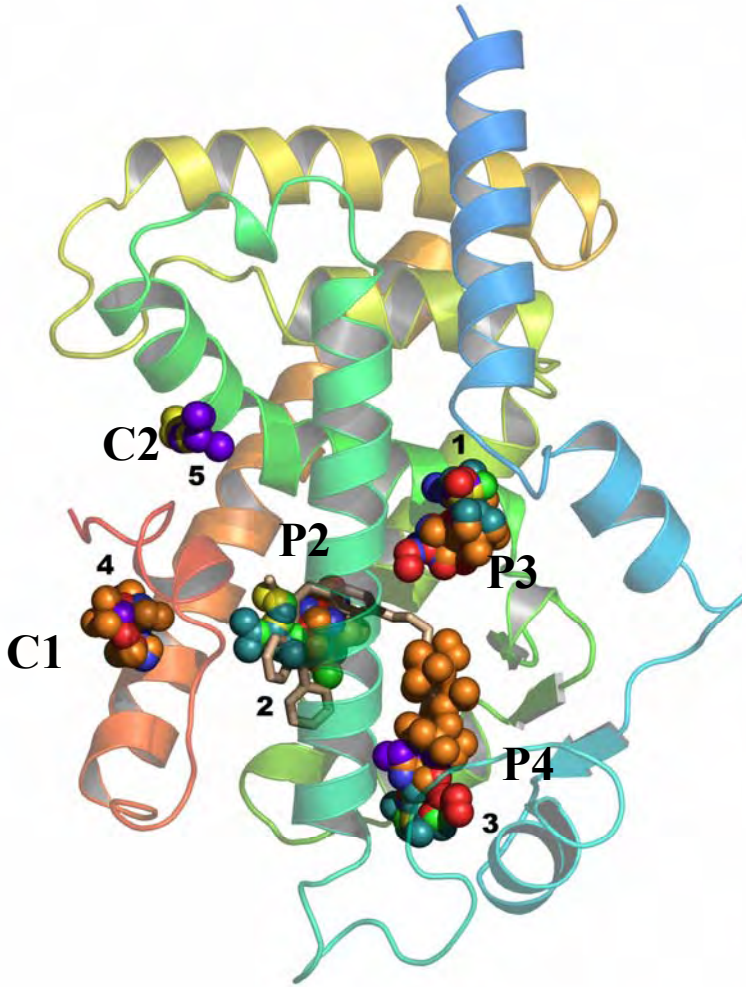
Dennis, S., Kortvelyesi T., and Vajda. S. Computational mapping identifies the binding site of organic solvents on proteins. *Proc. Natl. Acad. Sci. USA*., **99:** 4290-4295, 2002.

Kortvelyesi, T., Dennis, S., Silberstein, M., Brown III, L., and Vajda, S. Algorithms for computational solvent mapping of proteins. *Proteins.* **51:** 340-351, 2003.

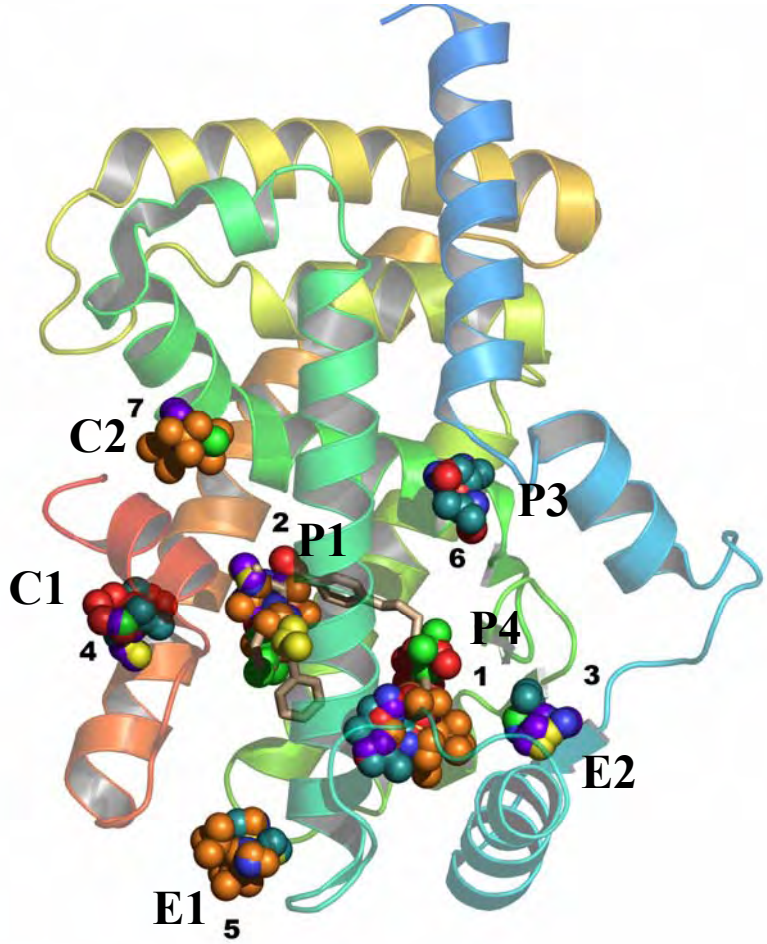
Silberstein, M., Dennis, S., Brown III, L., Kortvelyesi, T., Clodfelter, K., and Vajda, S. Identification of substrate binding sites in enzymes by computational solvent mapping, *J. Molec. Biol*. **332,** 1095-1113, 2003.

S. Vajda, 2007

Application to PPARγ



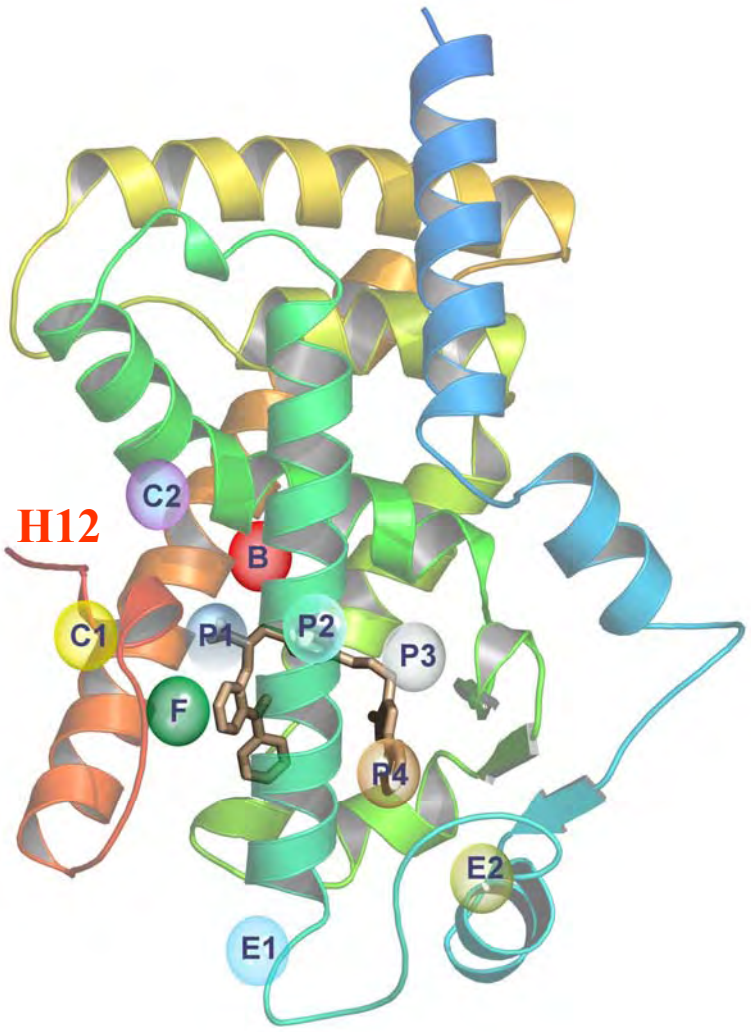
Unbound structure



Structure with farglitazar (1fm9)

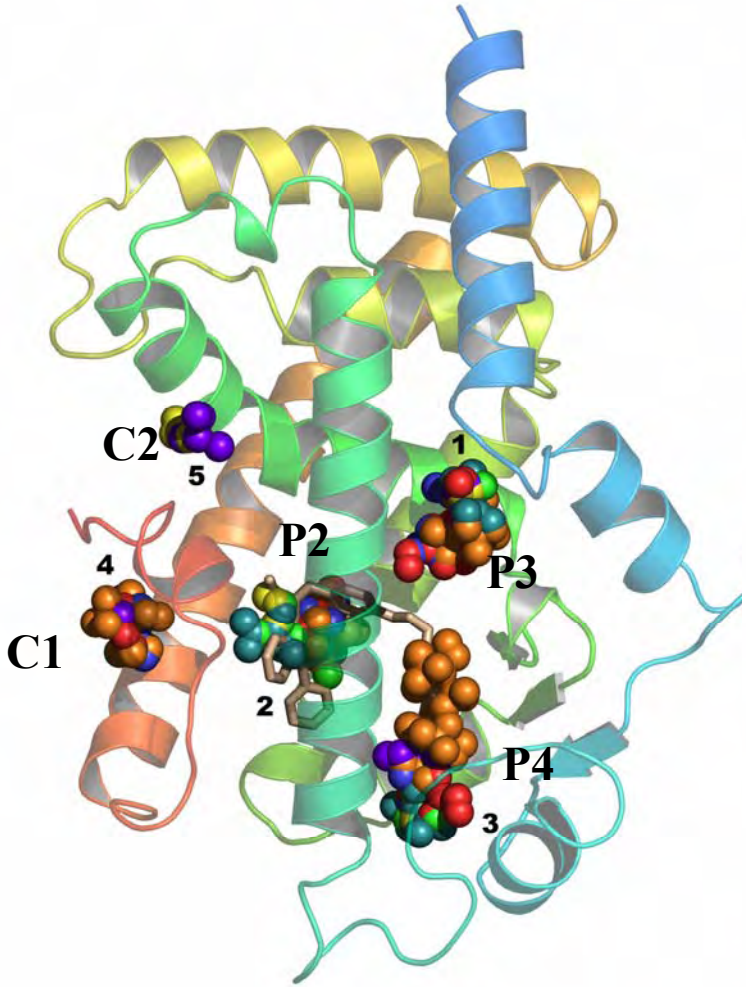
S. Vajda, 2007

Structure and “hot spots” of PPAR-γ

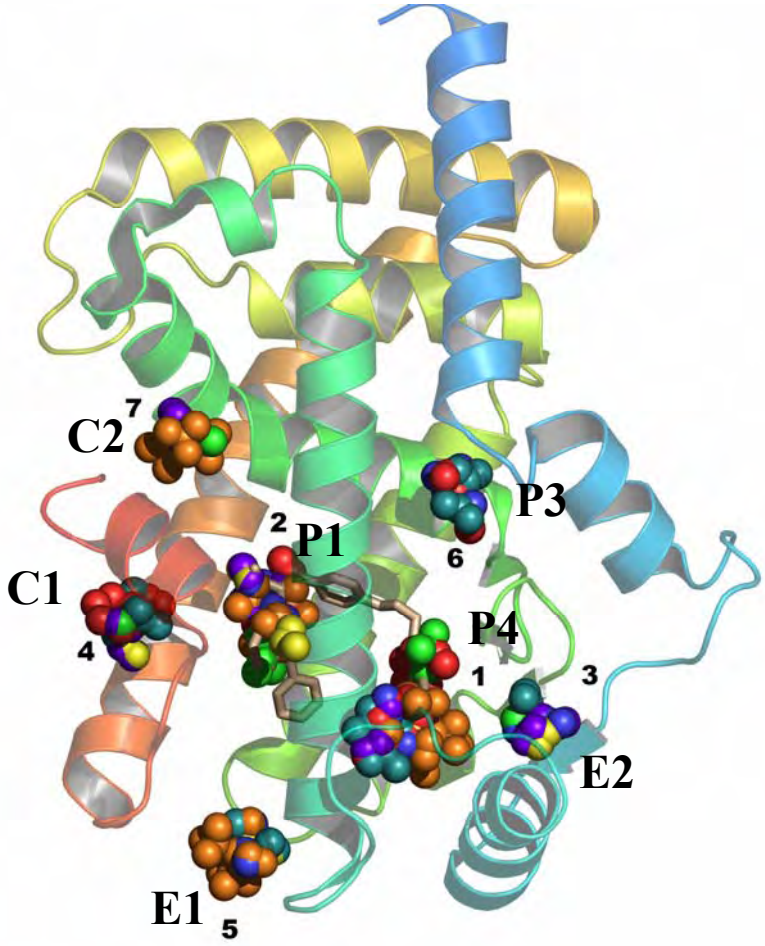


Site	Description
P1	Head group of agonists, interacting with helix H12
P2	Overlapping the middle of agonists
P3	Upper distal end of the binding site, reached only by the partial agonist
P4	Hydrophobic pocket close to the entrance
B	Surface pocket in the back, overlapping the dimerization region
F	Surface pocket of unknown role
C1	Surface pocket, possibly contributing to cofactor binding
C2	Overlapping with the binding site of the co-activator peptide SRC-1
E1	Pocket defined by the lower ends of helices H3, H7, and H10
E2	Putative ligand entrance between H2’ and the β-sheet

S. Vajda, 2007



Unbound structure



Structure with farglitazar (1fm9)

S. Vajda, 2007

Mechanistic insights

1. PPARγ binds a broad range of compounds:
   there is a well formed large binding site even in the ligand-free structure

2. Activation is selective:
   Only strong agonists can open pocket P1 and interact with H12.

3. Structurally similar PPARγ ligands can have significantly different pharmacological profiles:
   There are several co-activator binding regions (C1, C2, and F), and their shapes are affected by the structural details of the bound agonist, recruiting different coactivators.

Sheu, S-H., Kaya, T., Waxman D. J., and Vajda, S. Exploring the binding site structure of the PPARγ ligand binding domain by computational solvent mapping. Biochemistry, 44, 1193-1209, 2005

Conclusions from the mapping studies

1. Computational mapping finds the major subsites of ligand binding sites of proteins (so called “hot spots”)

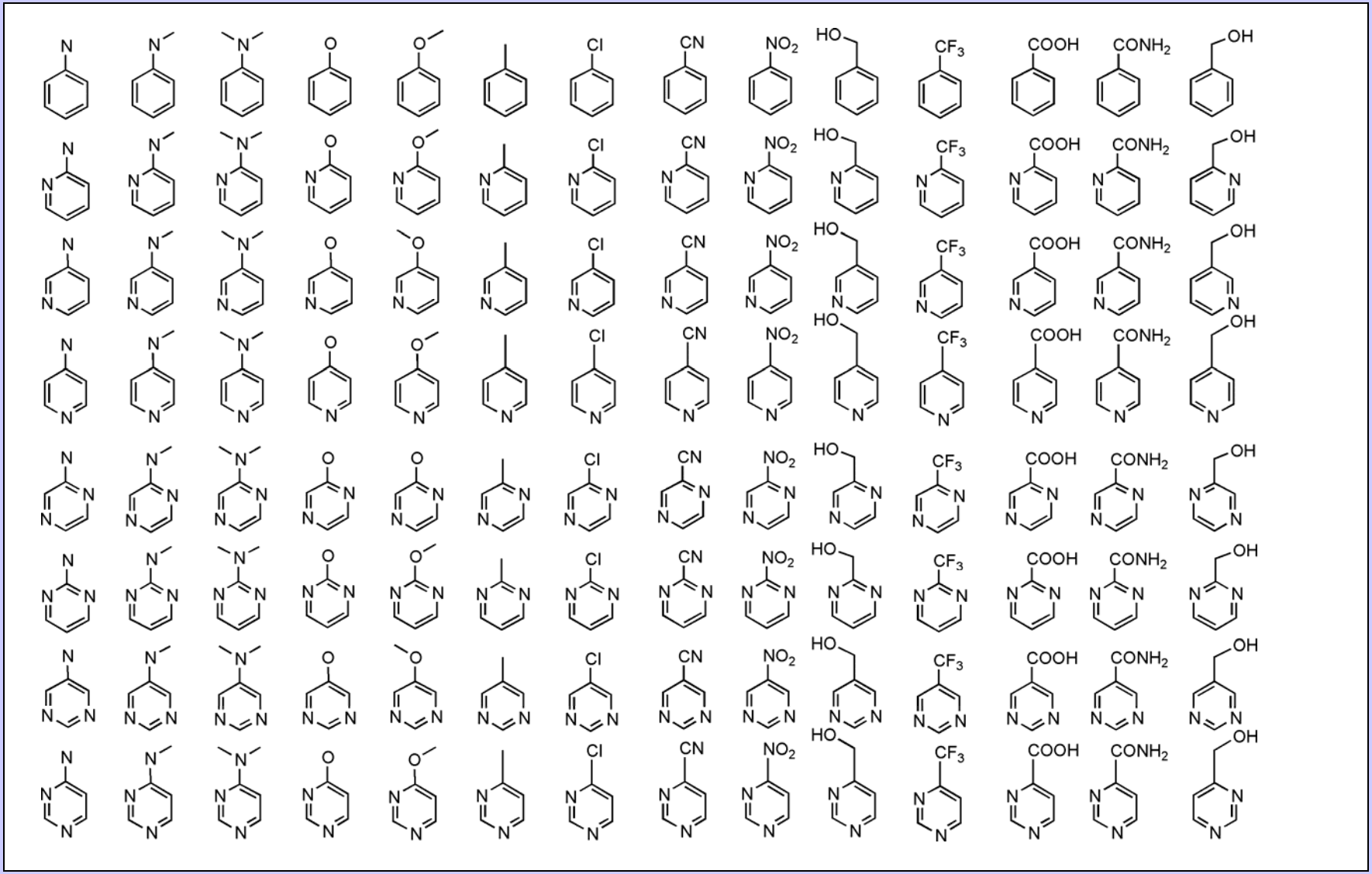
2. Mapping is a good tool to investigate the conformational changes that may affect the binding properties of various pockets

3. The low free energy and/or large clusters also identify the “hot spots” for specific functional groups.

Landon, M.R., Lancia, D.R. Jr., Yu, J., Thiel, S.C., and Vajda, S. Identification of hot spots within druggable binding sites of proteins by computational solvent mapping. *J. Med. Chem.,* **50:** 1231-1240, 2007.

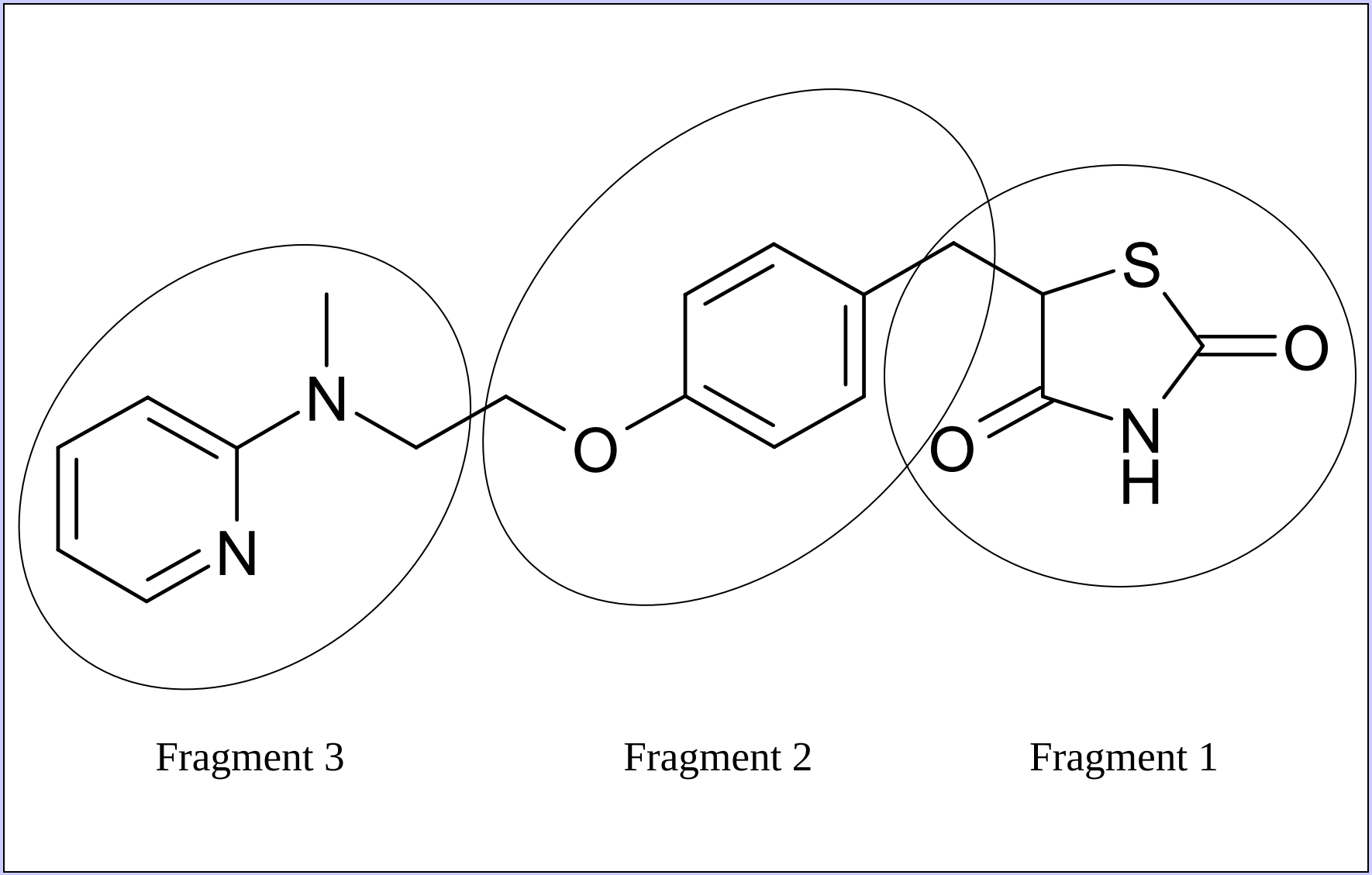
Vajda, S. and Guarnieri, F. Characterization of protein-ligand interaction sites using experimental and computational methods. *Current Opinion in Drug Design and Development.* **9:** 354-362, 2006.

Current work (a.k.a ideas and dreams): 1. Extended fragment libraries



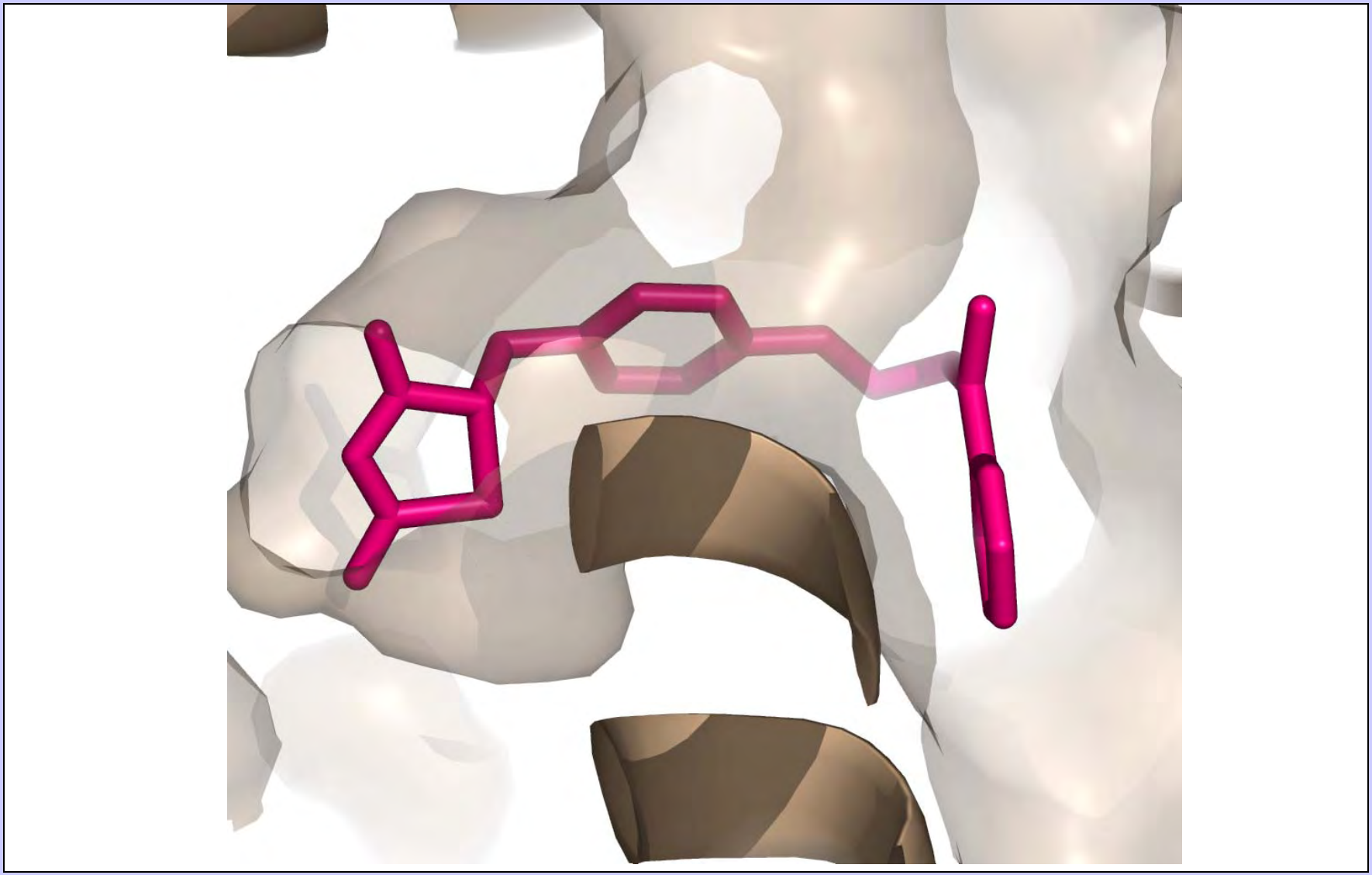
S. Vajda, 2007

Protein-specific fragment libraries: (e.g. rosiglitazone fragments)



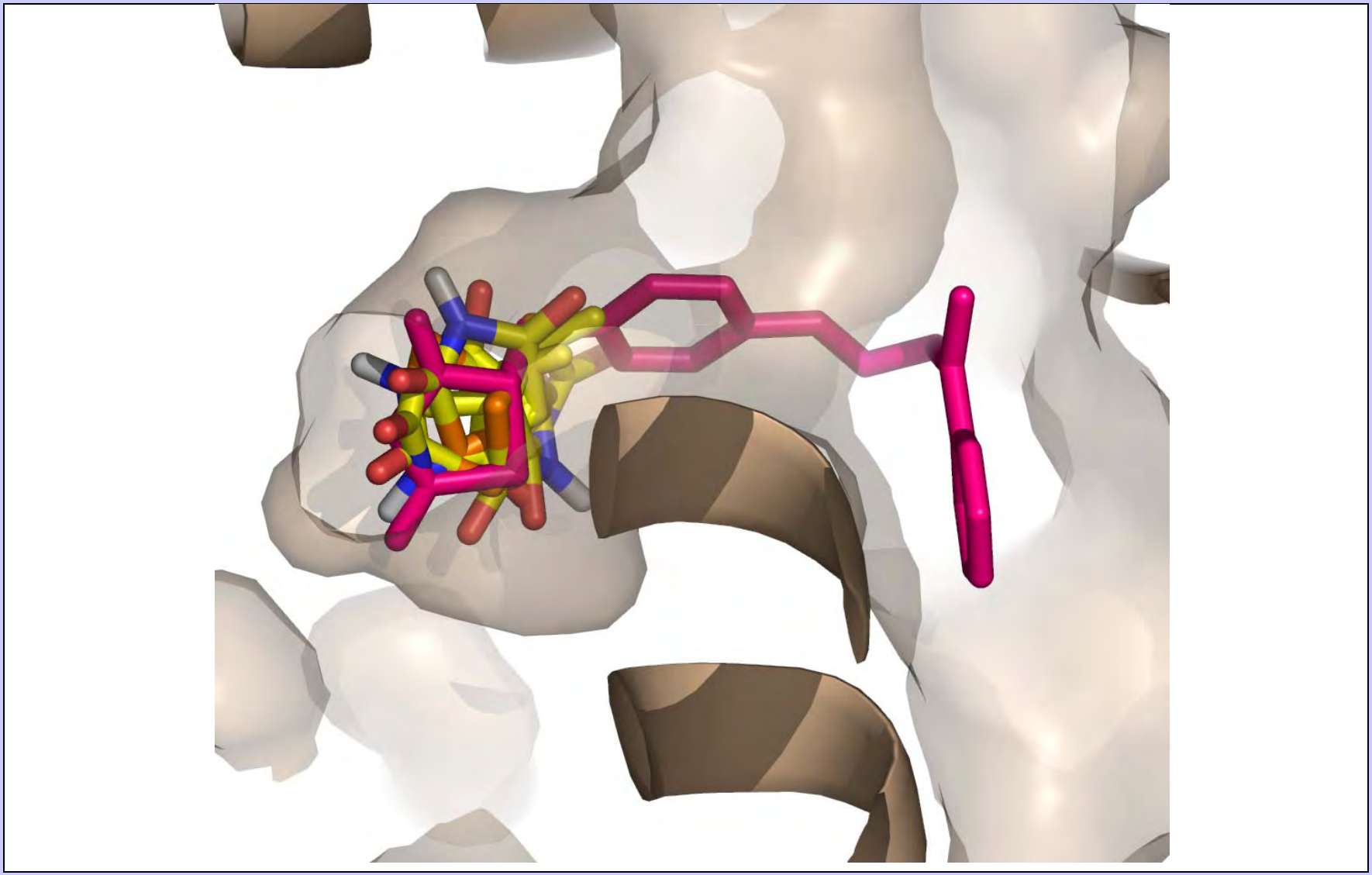
S. Vajda, 2007

Rosiglitazone bound to PPARγ



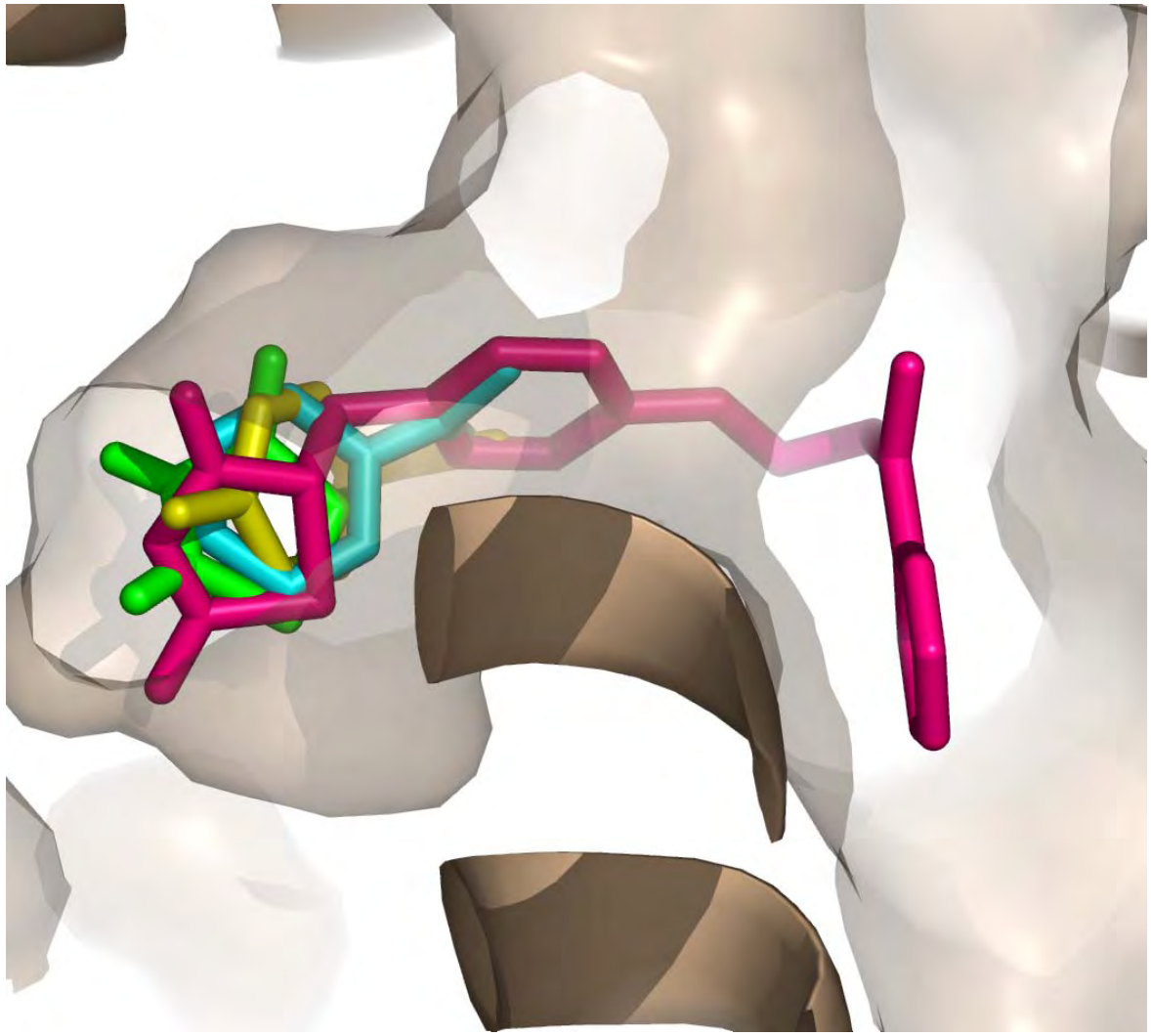
S. Vajda, 2007

Lowest energy and large cluster of the TZD group is in the AF-2 region



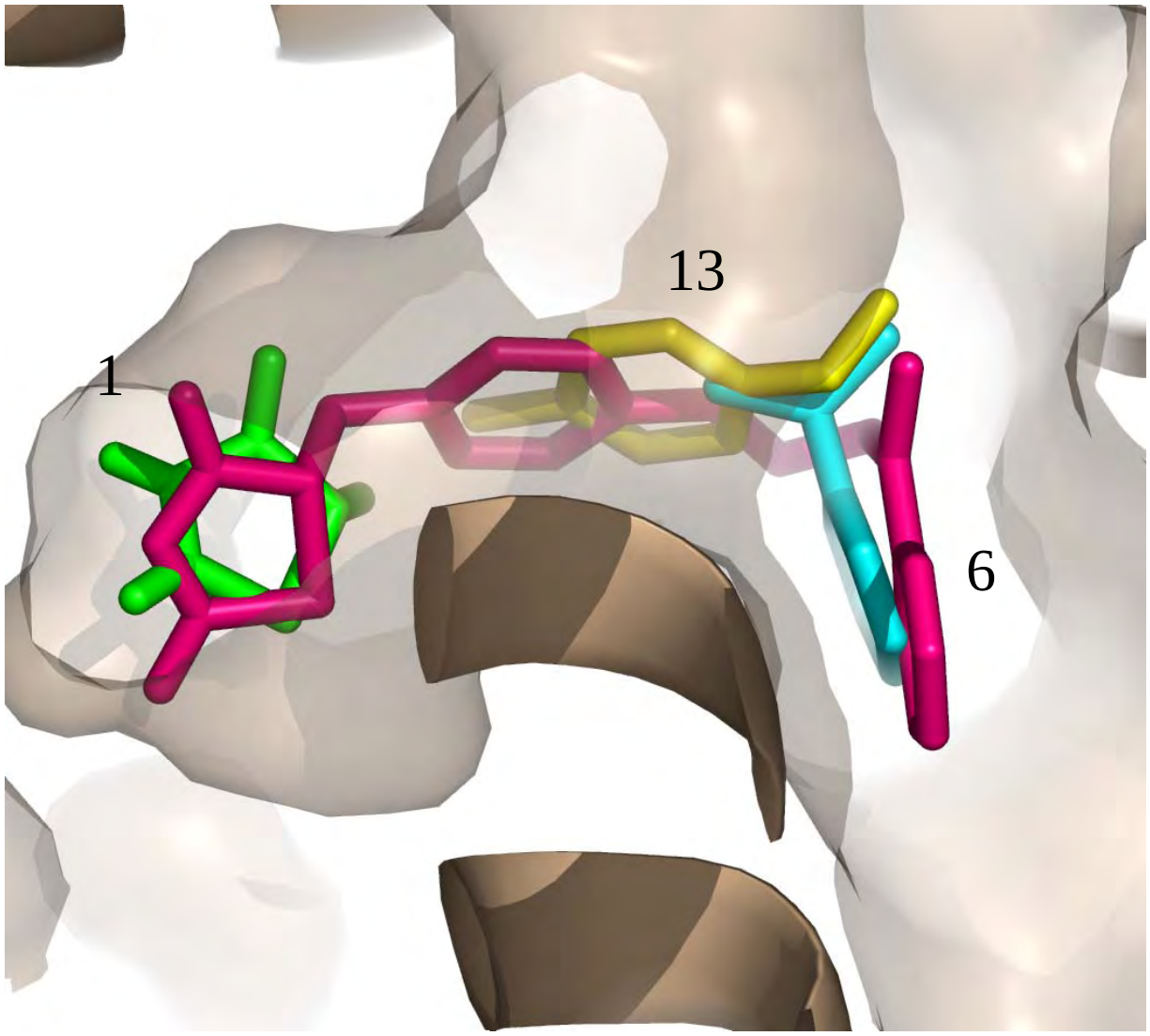
S. Vajda, 2007

Other types of rings also bind there, but form smaller clusters



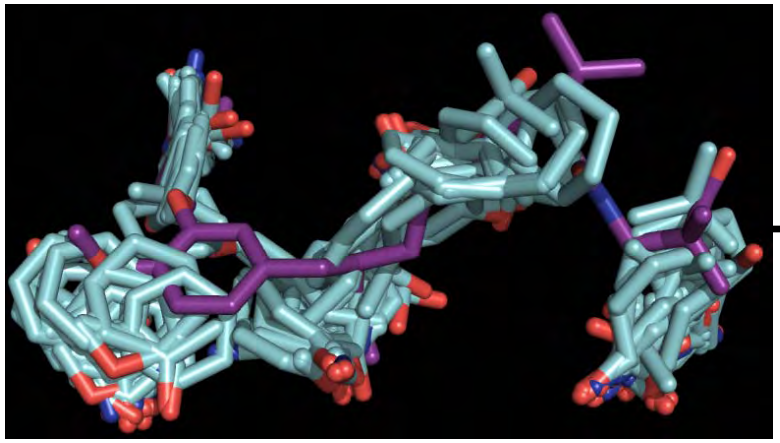
S. Vajda, 2007

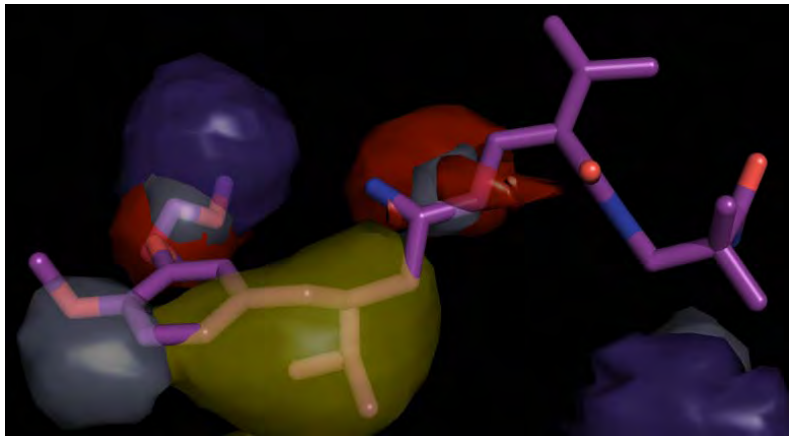
Most fragments also find their own positions at higher energy



S. Vajda, 2007

3. Atom type-based density characterization of hot spots





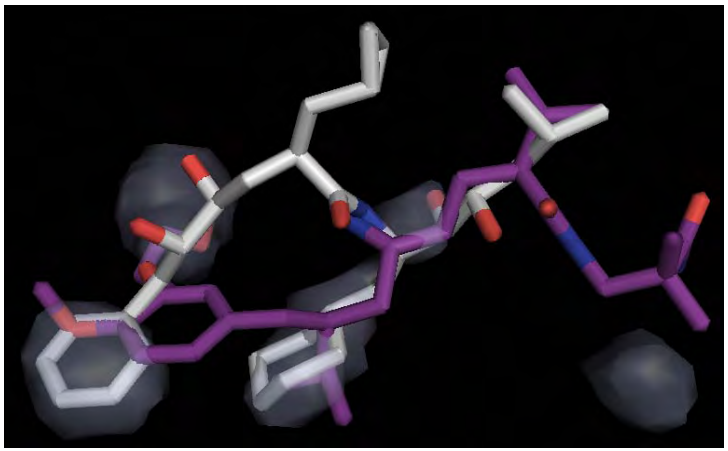
Renin with a new drug, Aliskiren plus low energy probes from the mapping

Pharmacophore representation yellow: aromatic carbon, grey: aliphatic carbon, red: oxygen, blue: nitrogen

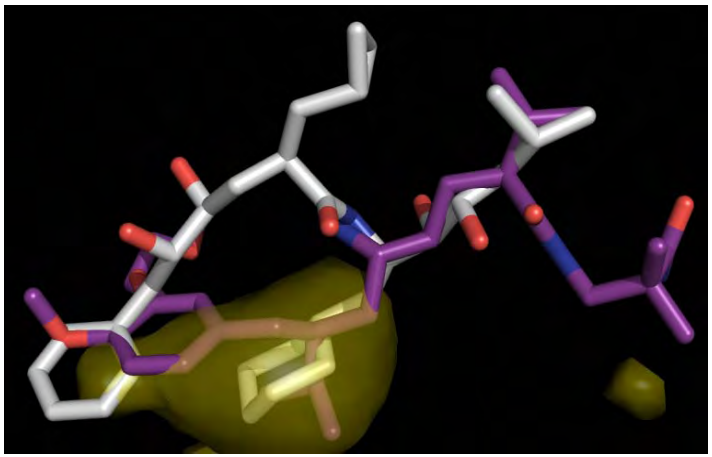
Landon, M.R., Lancia, D.R. Jr., Yu, J., Thiel, S.C., and Vajda, S. Identification of hot spots within druggable binding sites of proteins by computational solvent mapping. *J. Med. Chem.,* **50:** 1231-1240, 2007.

Example: Renin with Aliskiren and a peptidomimetic

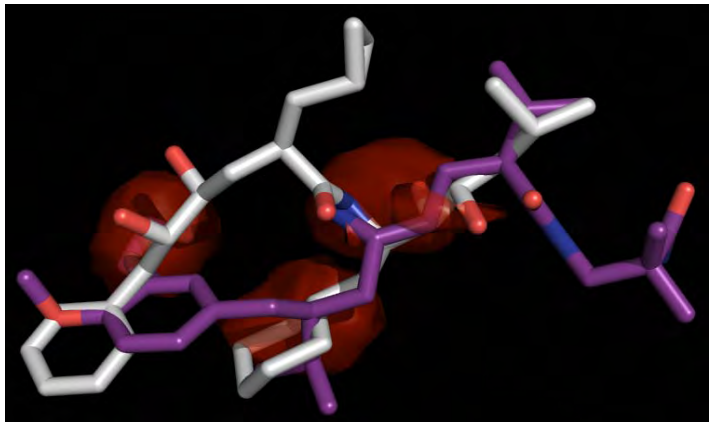
Aliphatic carbon



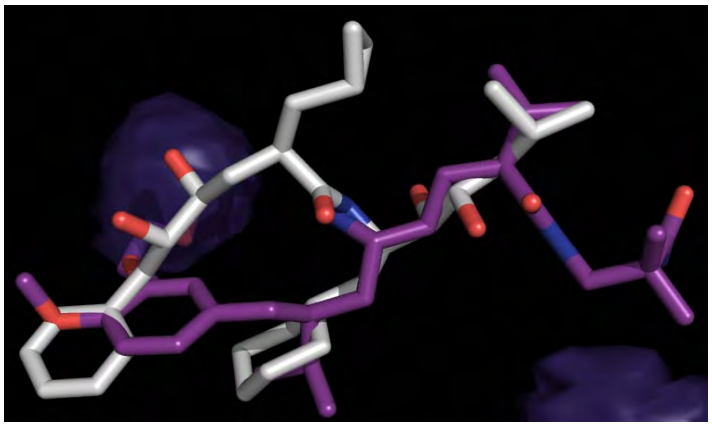
Aromatic carbon



Oxygen



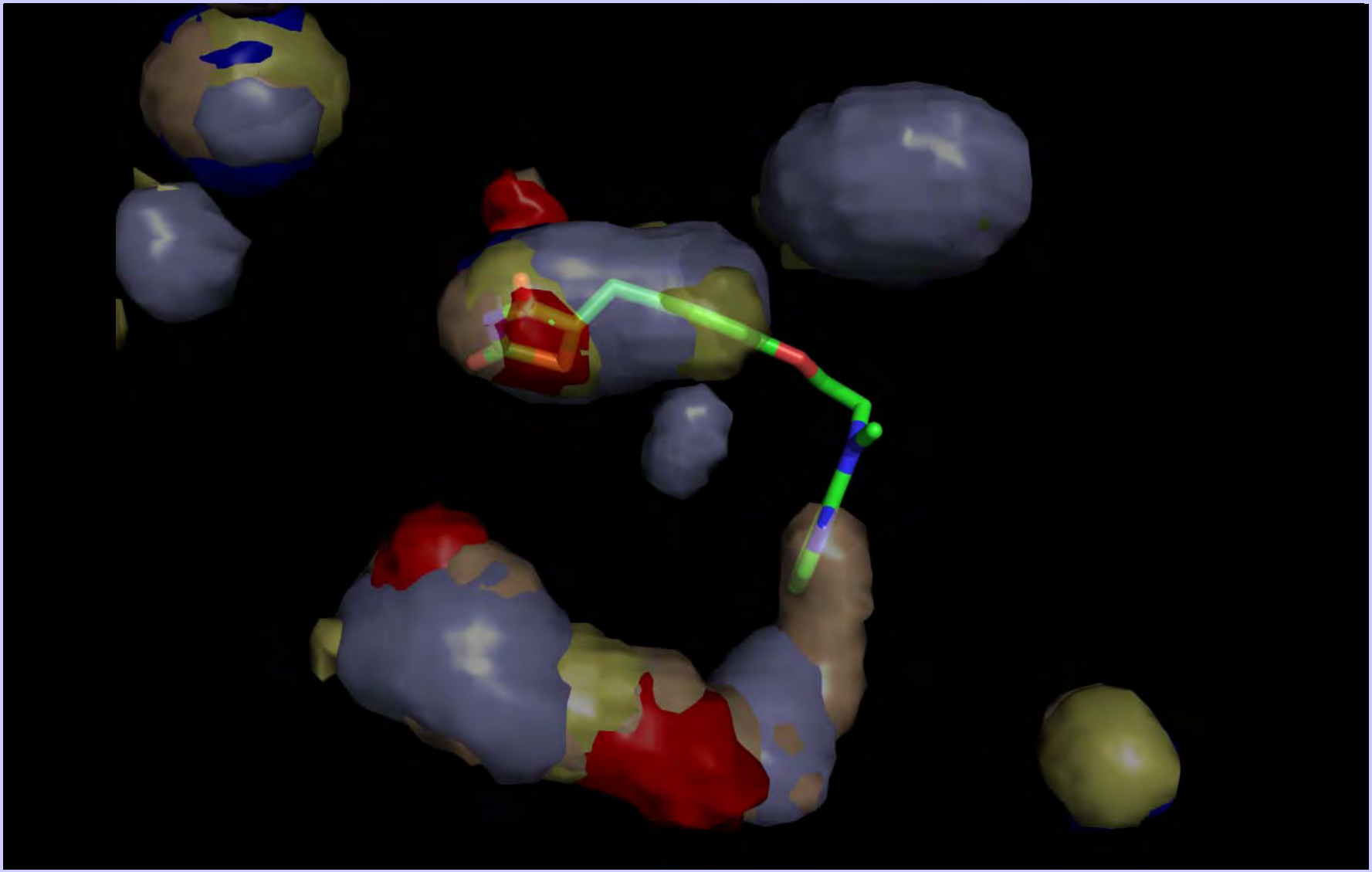
Nitrogen



Aliskiren Score = 516.7, Peptidomimetic Score = 337.2

S. Vajda, 2007

Preliminary results for PPARγ



S. Vajda, 2007

Ideas

- Develop libraries of fragments from a number of xenobiotics, and map a set of potentially interacting proteins
- Find the lowest energy clusters of specific functional groups and use them for the construction of (structure-based) “pharmacophores”
- Screen larger database of small molecules for potential interactions with the proteins by fitting the molecules into the pharmacophores derived by the mapping

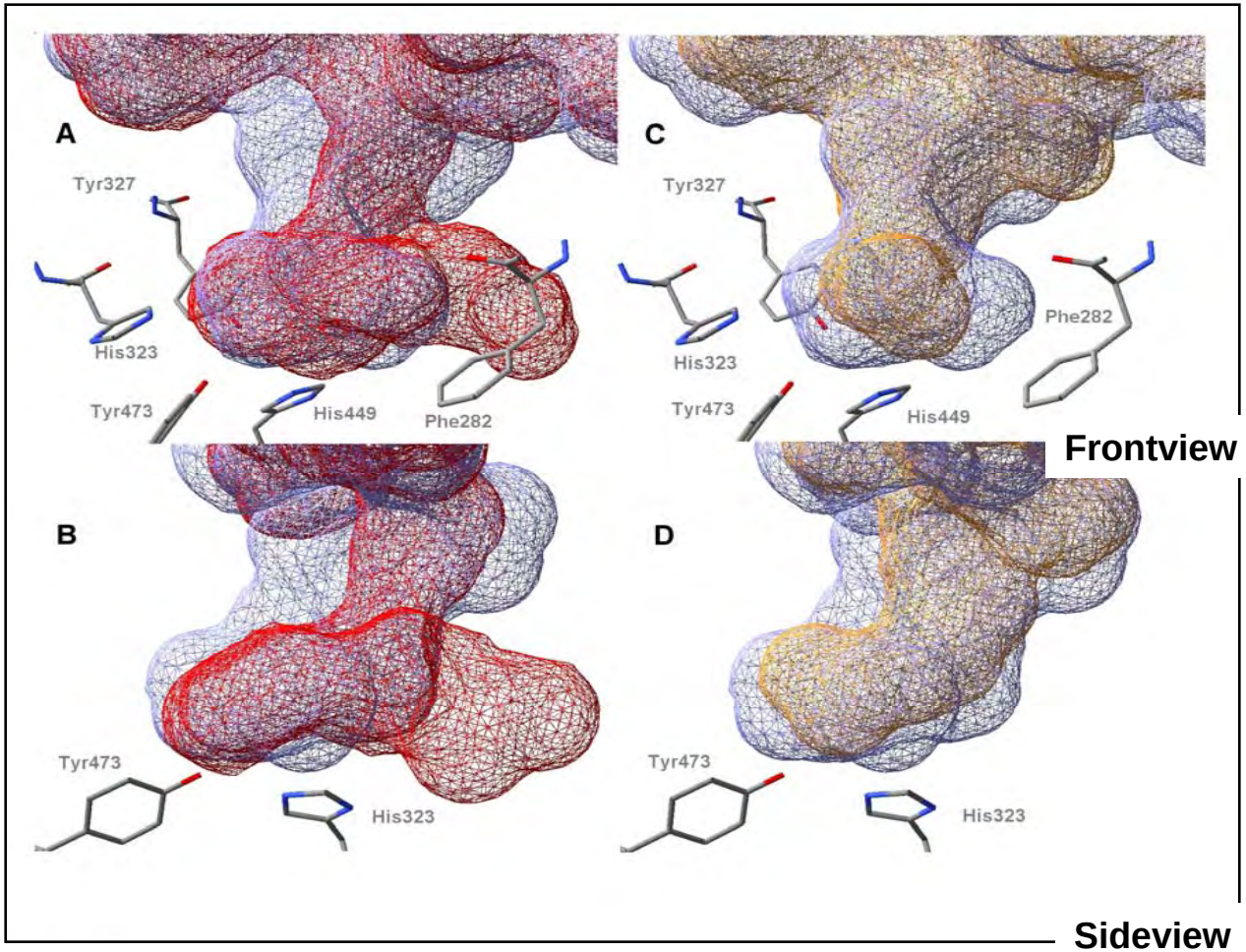
Credits

Dr. Sheldon Dennis
Dr. Lawrence Brown
Dr. Tamas Kortvelyesi
Shu-Hsien Sheu
David Lancia
Michael Silberstein
Karl Clodfelter
Spencer Thiel
Melissa Landon
Dima Kozakov

Dr. Dagmar Ringe (Brandeis University)
Dr. David Waxman (Boston University)
Dr. Patrick Griffin (Scripps Florida)

National Institute of Health
National Institute of Environmental Health
National Science Foundation
ExSAR Corporation
SolMap Pharmaceuticals

Change in the P1 pocket



Comparison of the pockets in ligand-free, Rosiglitazone-bound, and partial agonist-bound structures

S. Vajda, 2007

Details of our CS-Map algorithm

<u>Step 1:</u> Rigid body search: Move (rigid) ligand molecules using a multi-start nonlinear simplex method in the electrostatic and desolvation field of the protein toward positions with low values of the simplified free energy expression

$$\Delta G_s = \Delta E_{elec} + \Delta G_{des} + V_{exc}$$

where

$\Delta E_{elec} = \Sigma\Phi(x_i)q_i$ Quasi-Coulombic approximation

ΔG_{des} Calculated using a pairwise structure-based potential

V_{exc} Excluded volume term, >0 only if there is overlap.

200 to 300 initial positions distributed on the protein surface, 30 simplex runs from each with random initial orientation of the simplex, resulting in 6000 to 9000 minima.

<u>Step 2:</u> Flexible search: Minimize the complexes derived in Step 1 using the more accurate free energy function

$$\Delta G = \Delta E_{elec} + \Delta G^*_{des} + \Delta E_{vdw}$$

where ΔG^*_{des} includes the solvent-solute van der Waals interactions.

$\Delta E_{elec} + \Delta G^*_{des}$ Analytical Continuum Electrostatics: Generalized Born model

ΔE_{vdw} 6-12 Lennard-Jones potential

The local minimization allows for the flexibility of the probe molecules. Performed at 6000 to 9000 local minima.

S. Vajda, 2007

The CS-Map algorithm: continuation

Step 3: Cluster the solutions on the basis of pairwise distances. Remove small clusters (with less than 15-20 minima).

Step 4: Rank clusters on the basis of average free energy:
Calculate the (Boltzmann) average free energy of each clusters by

$$\langle \Delta G \rangle_i = \Sigma\, p_j\, \Delta G_j$$

where

$$p_j = \exp(-\Delta G_j/RT)/\{\Sigma \exp(-\Delta G_j/RT)\}$$

Step 5: Identification of consensus sites: Use the 5 lowest free energy clusters of each ligand. Consensus sites are the positions at which several of such clusters of different probes overlap. The consensus sites ranked on the basis on the number of clusters included.

Step 6: Testing the stability of bound states by molecular dynamics